

## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2021 11 18 - pfizer\13-13-43\2021 11 18 13H 13M.raw  
**Created:** Thursday, November 18, 2021 1:39:17 PM  
**Number of capillaries:** 12  
**Array serial number:** 022621-27SFS  
**Effect length:** 33 cm  
**Array usage count:** 35  
**Instrument type:** 5300 Fragment Analyzer  
**Instrument controller software version:** 3.1.0.12  
**Device serial number:** MY2105AB19

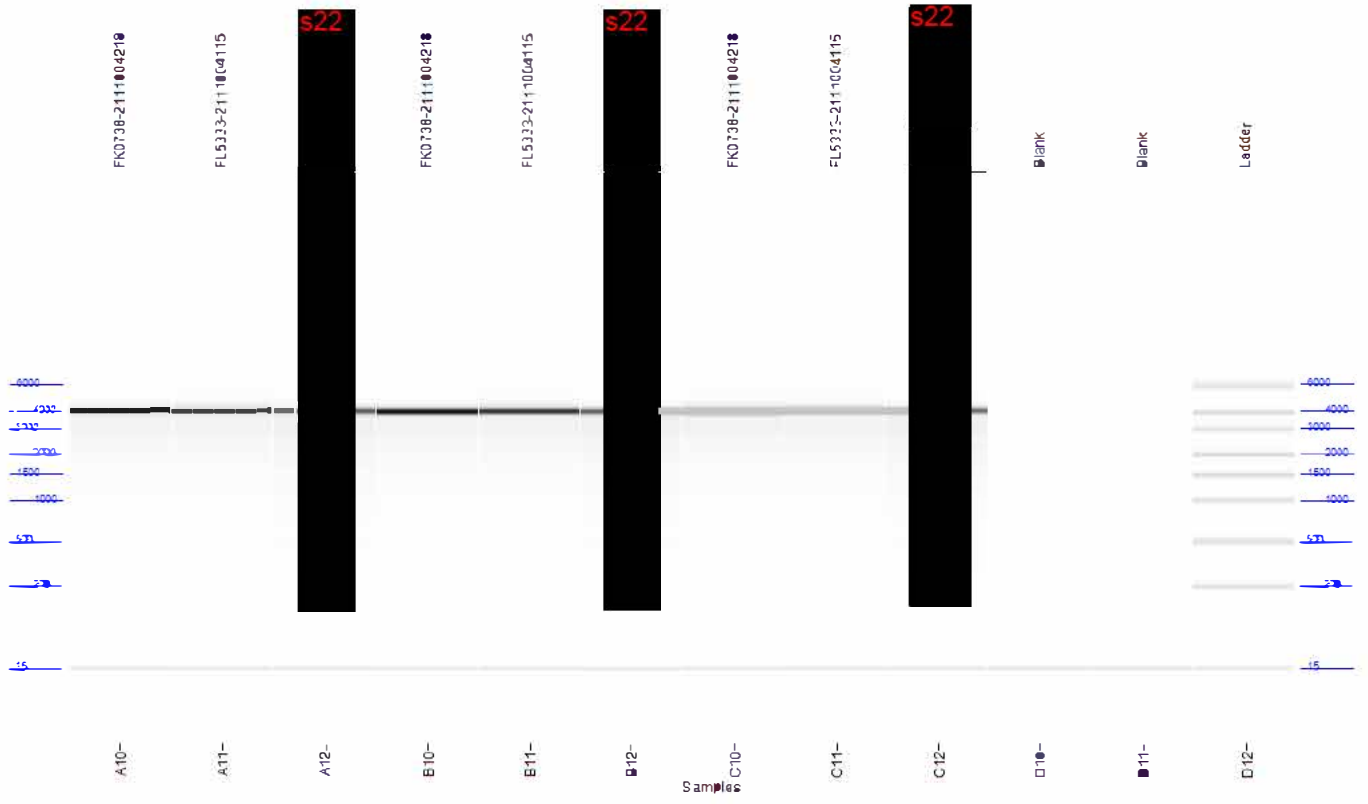
## Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds  
**Gel prime:** No  
**Full conditioning:** Yes  
**Gel prime to buffer:** Yes  
**Gel selection:** Gel 2  
**Perform prerun:** 8.0 kV, 30 sec.  
**Rinse:** No  
**Marker 1:** No  
**Rinse:** Tray: 3, Row: A, Dip count: 2  
**Sample injection:** 5.0 kV, 6 sec.  
**Separation:** 8.0 kV, 60.0 min.  
**Tray name:** Tray-1

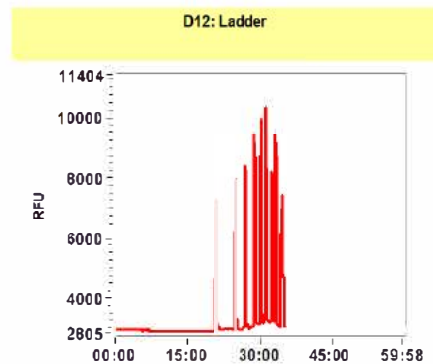
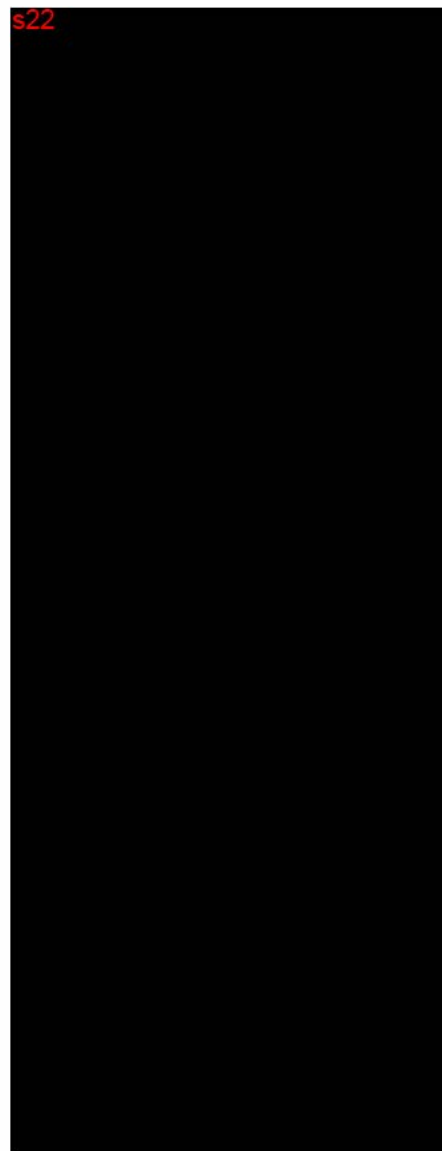
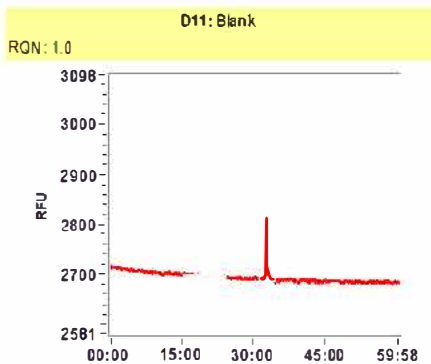
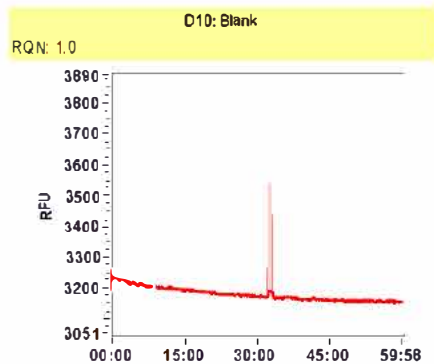
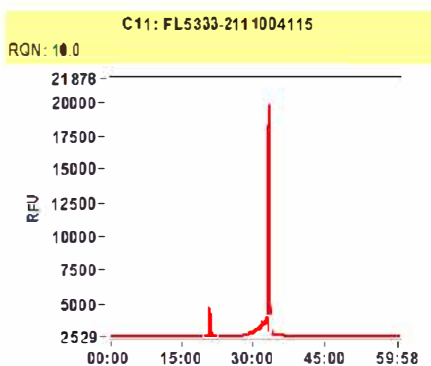
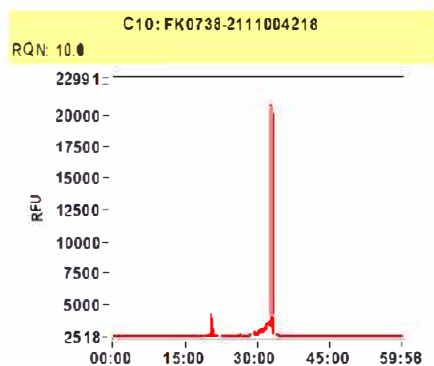
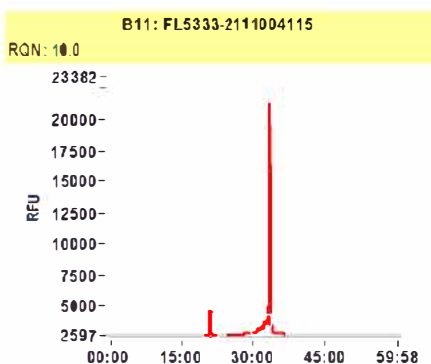
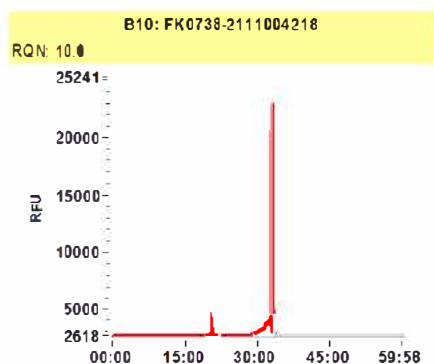
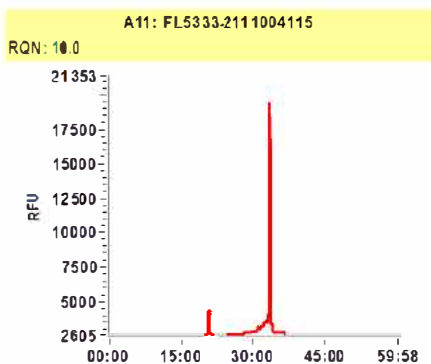
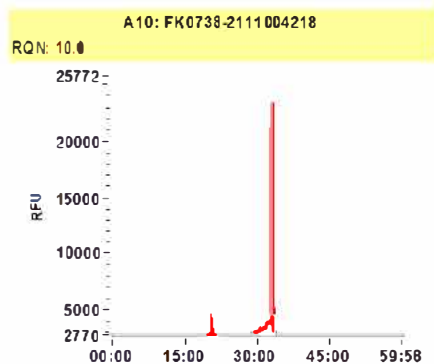
**Analysis mode:** RNA (Eukaryotic)

## Notes

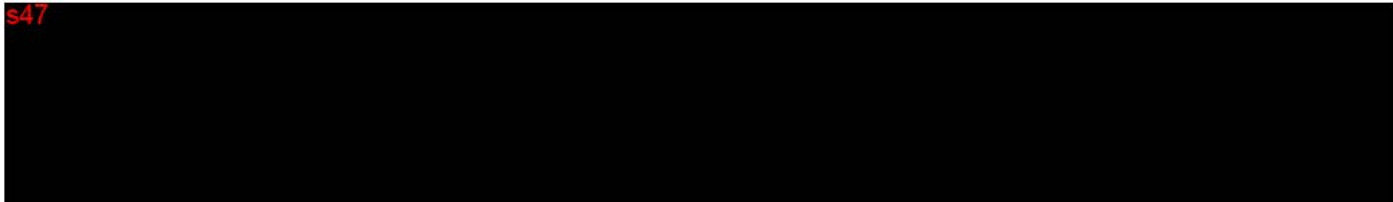
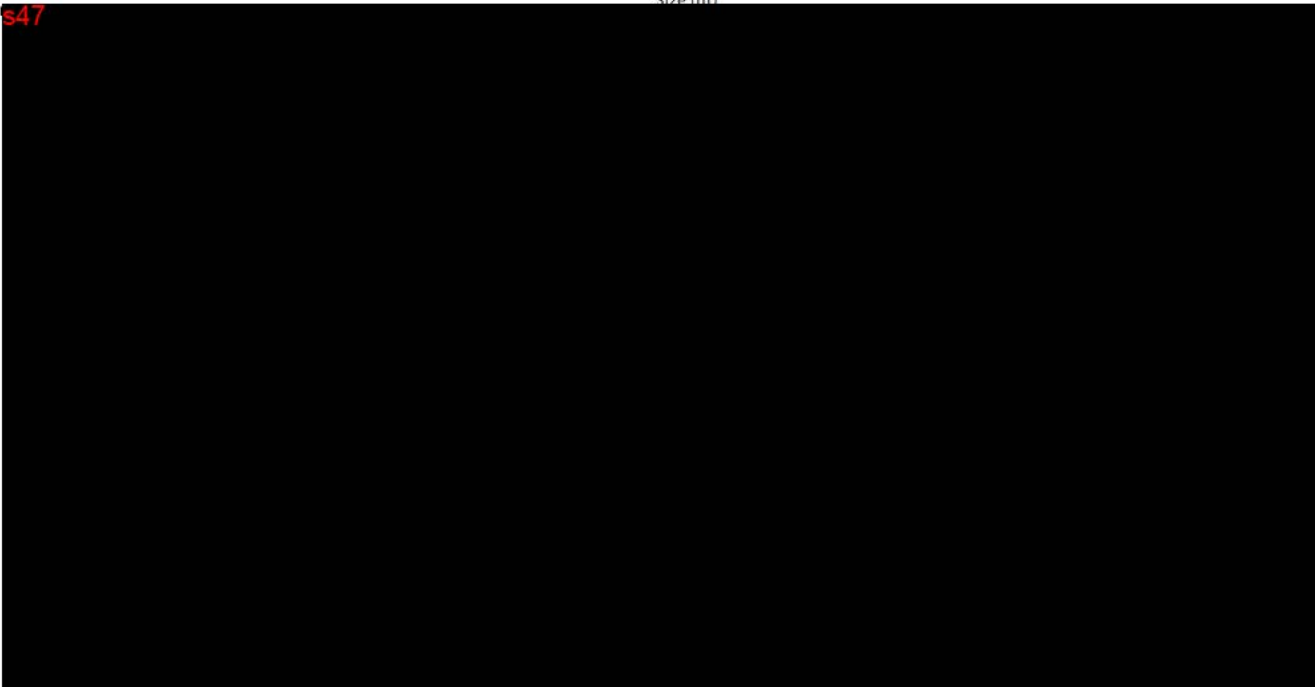
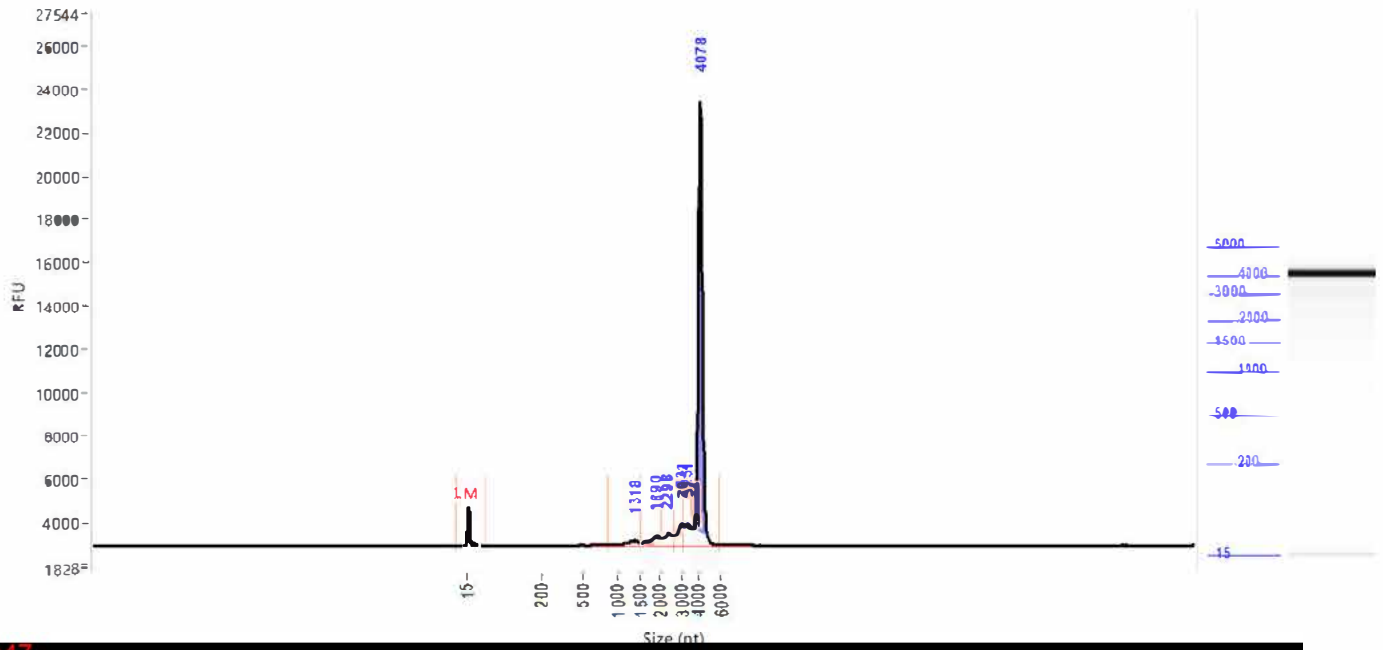
# Gel Image



s22

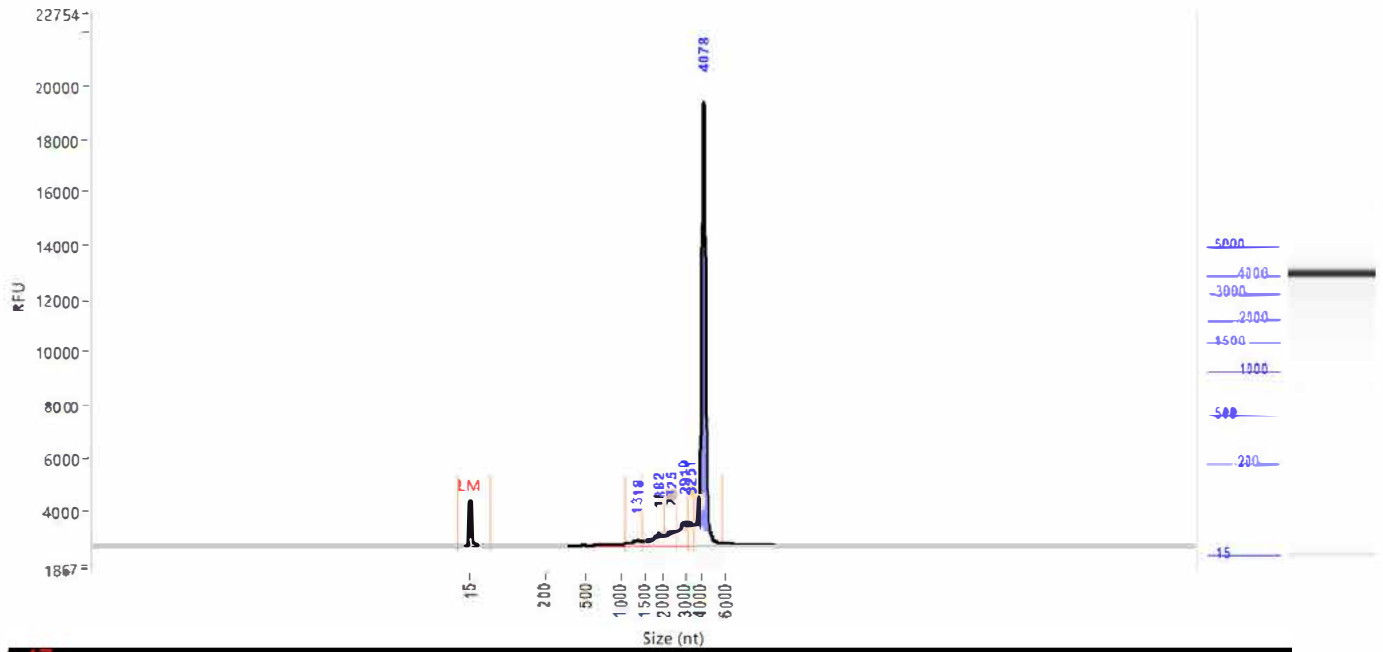


Sample: EK0738-21111004218



Sample: FL5333-2111004115

s22



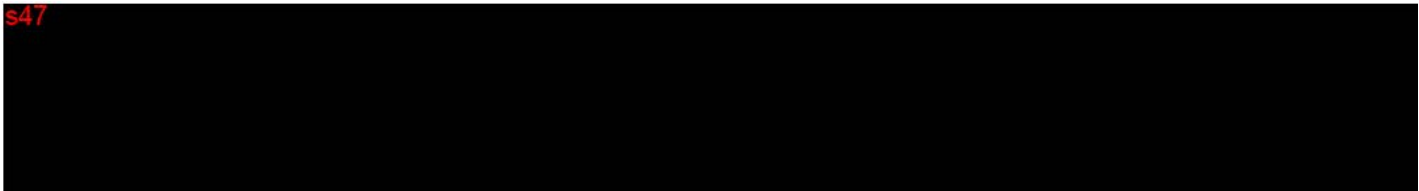
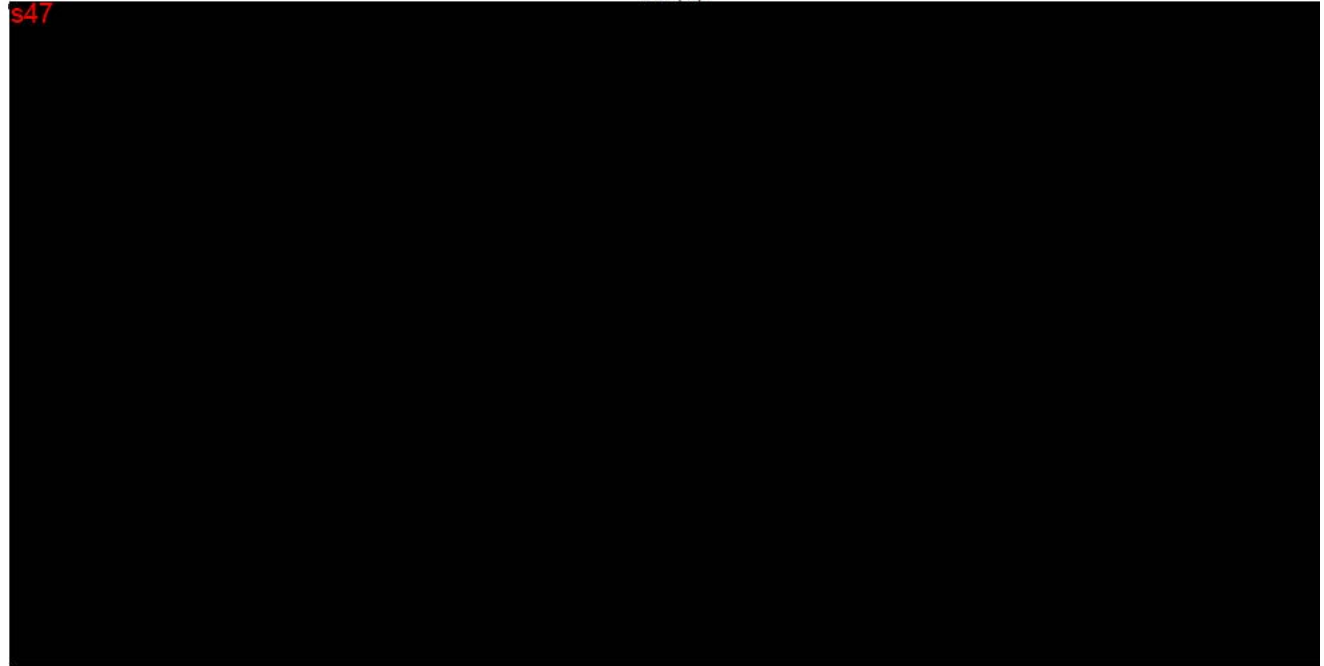
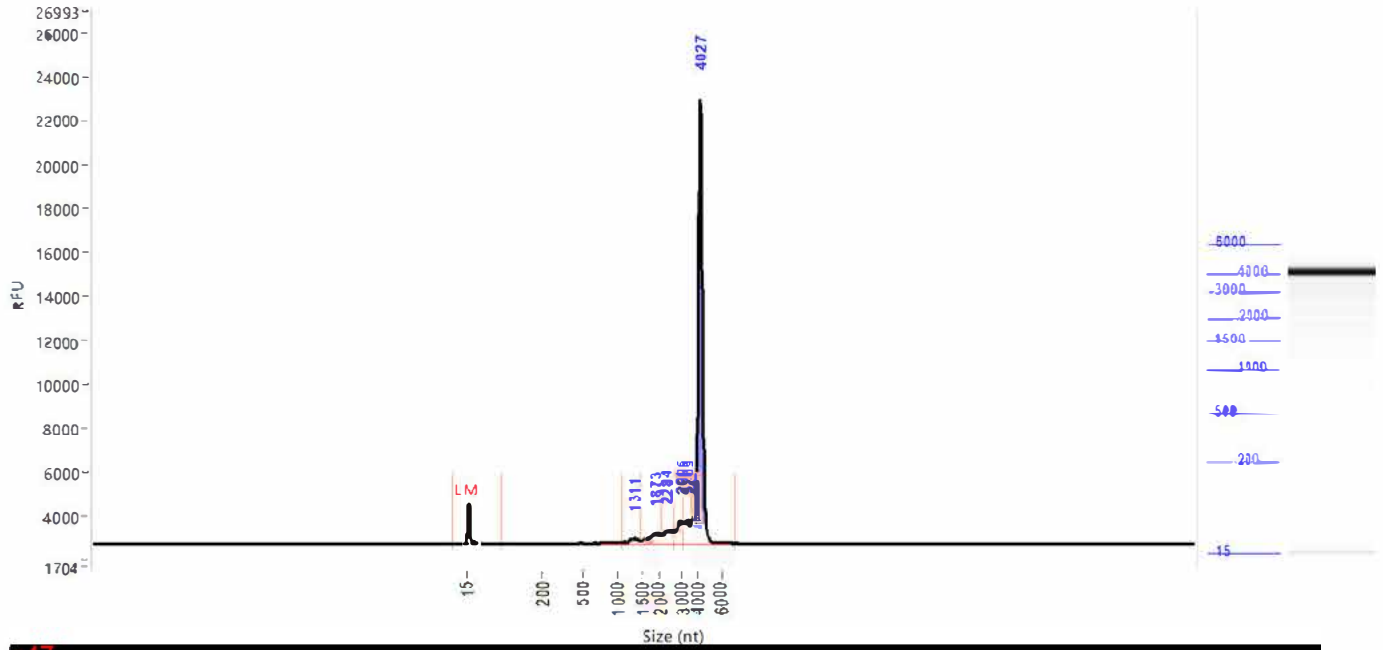
s47

s47

Sample **s22**  
**s22**

**s22**

Sample: FK0738-21111004218

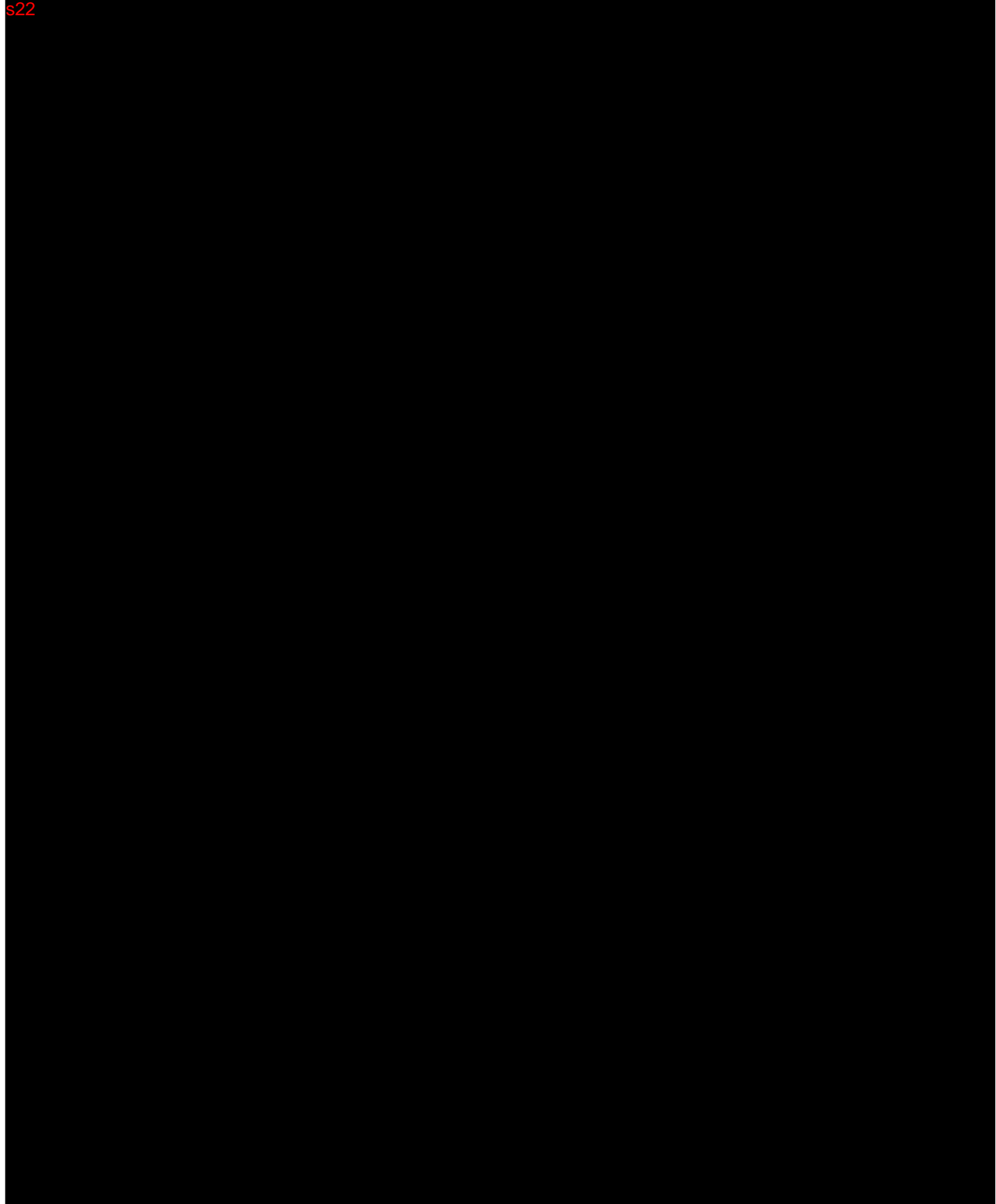


**Sample:** FL5333-2111004115  
s22

s47

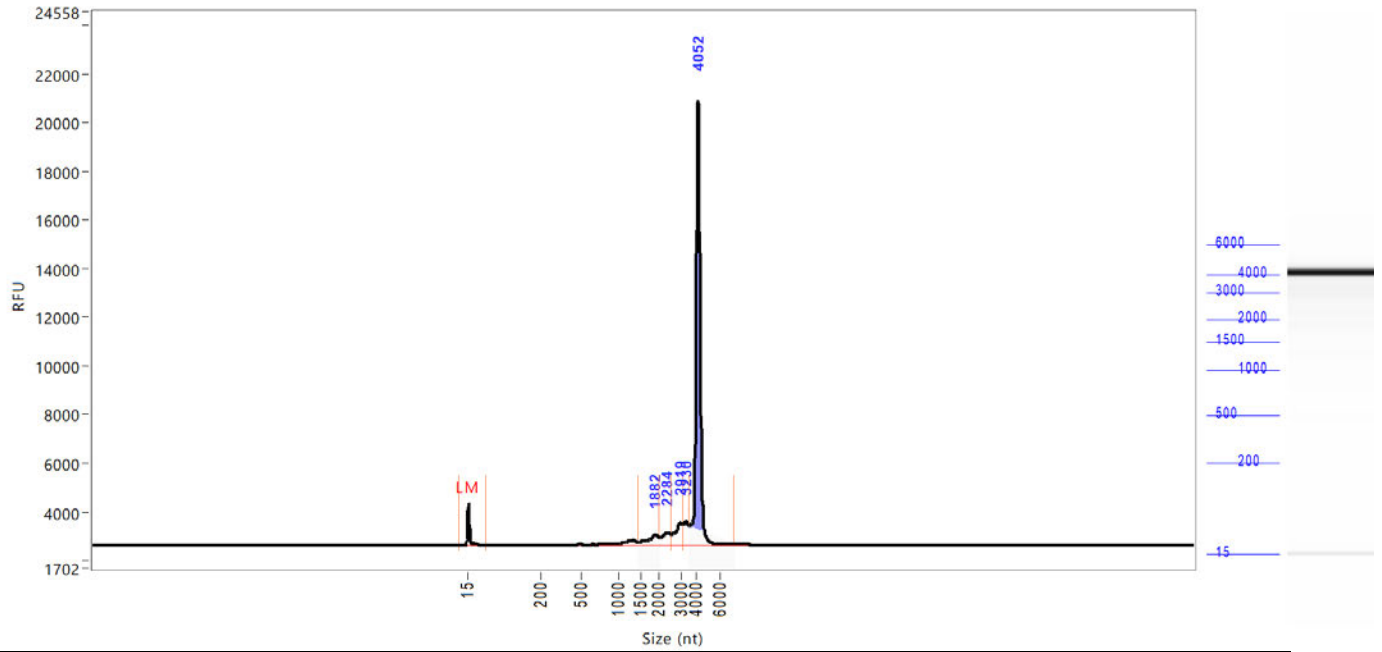
s47





Sample: FK0738-2111004218

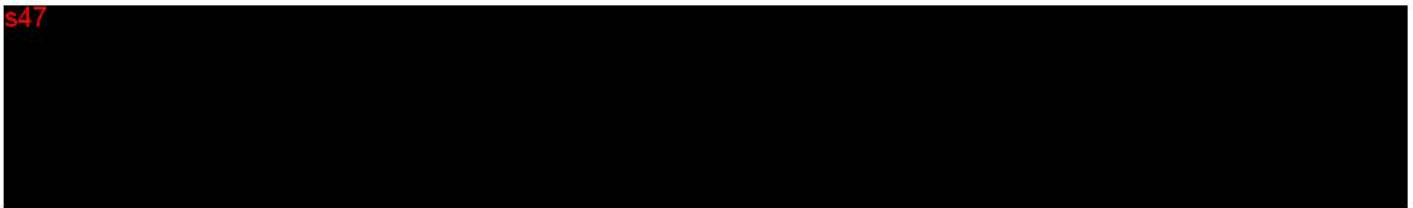
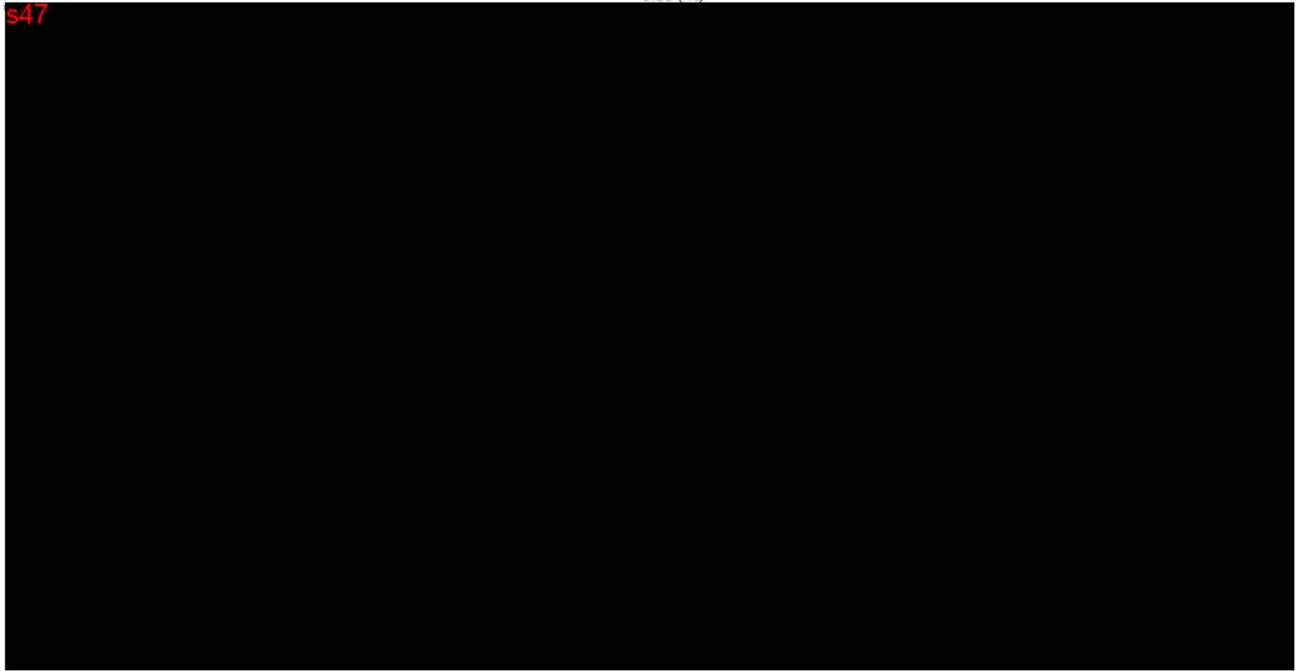
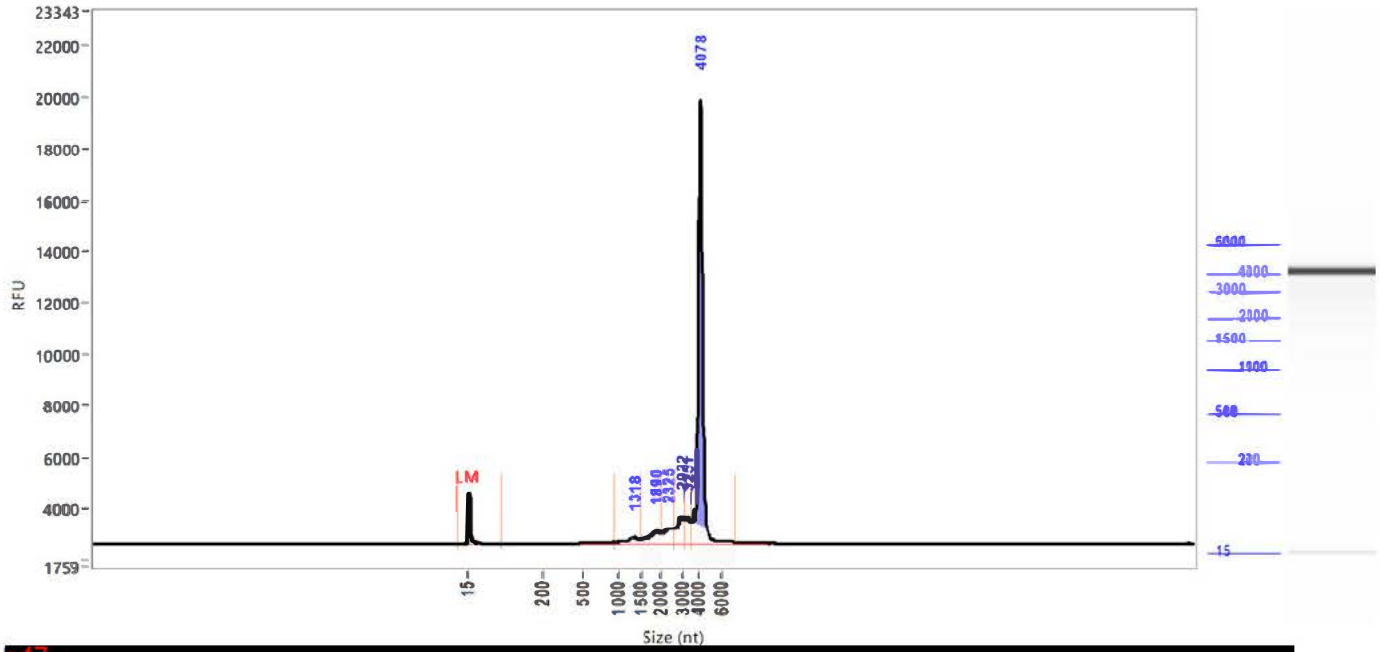
s22



s47

s47

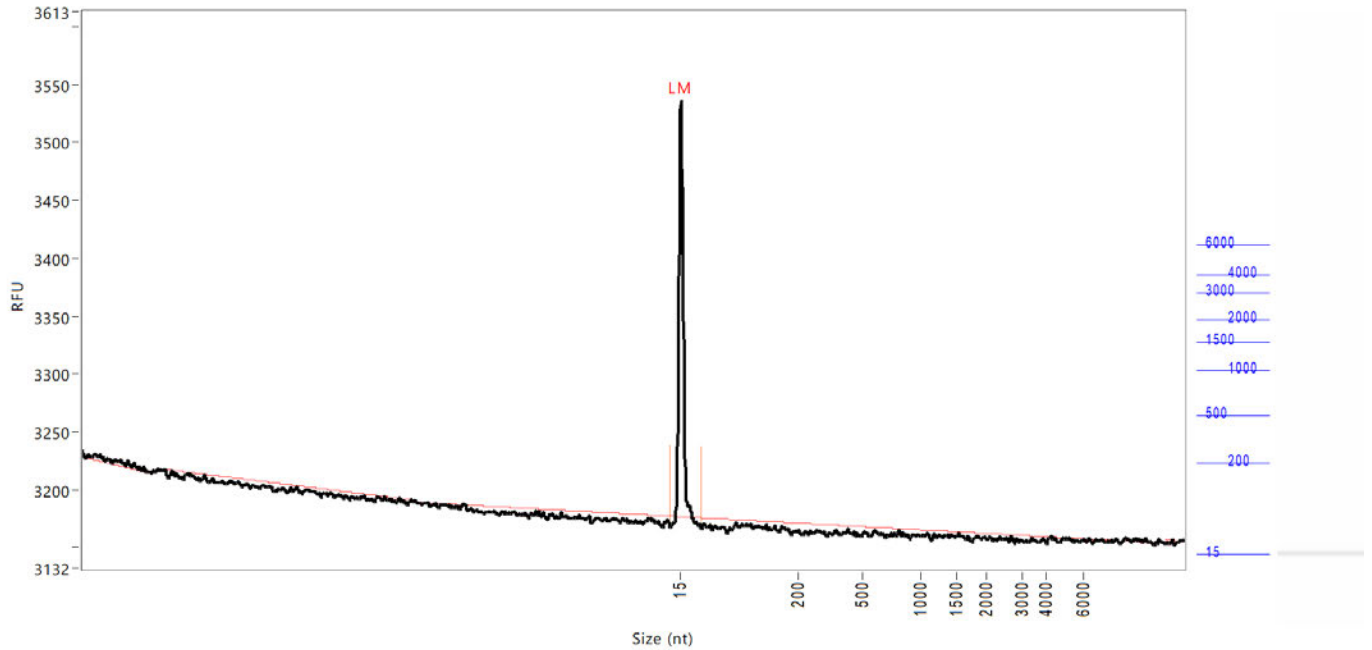
Sample: FI 5333-2111004115



s22



**Sample:** Blank  
**Well location:** D10  
**Created:** Thursday, November 18, 2021 1:39:17 PM

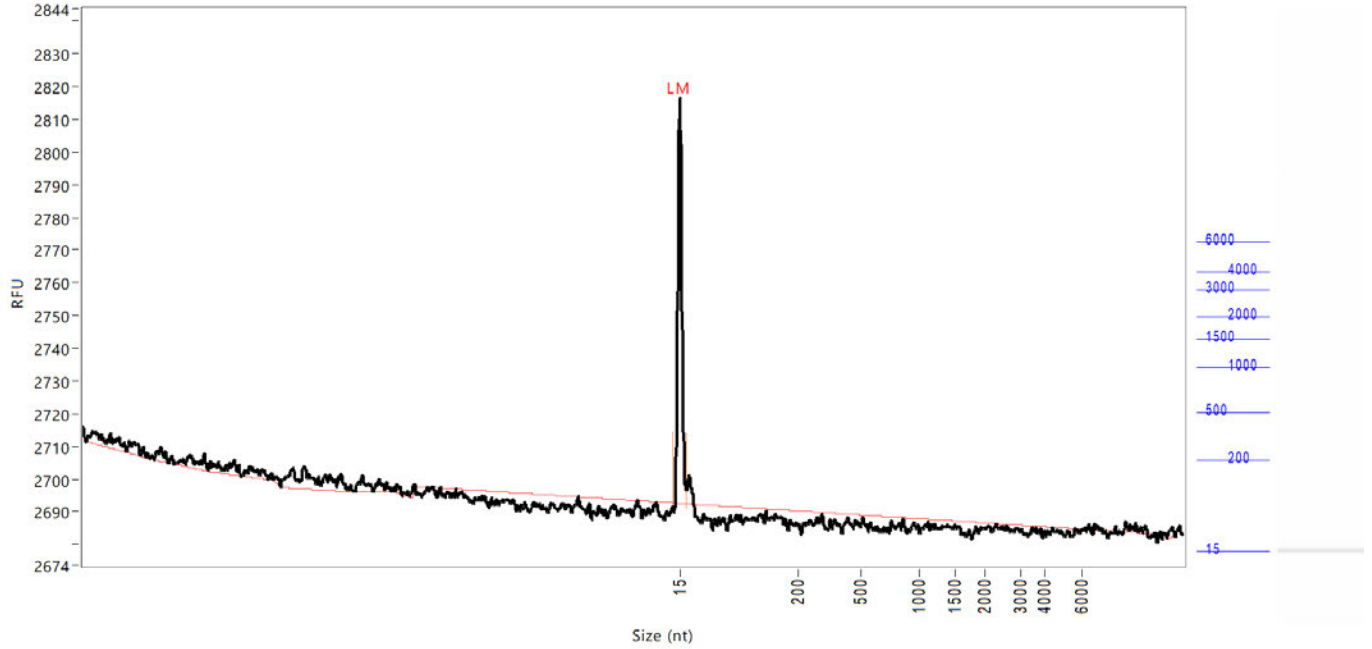


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.7137	0	48	358
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0162	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0162 ng/ul	100.0 %Total	0.0055 nmole/L	9147 Avg. Size (nt)	7.07 %CV

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Blank  
**Well location:** D11  
**Created:** Thursday, November 18, 2021 1:39:17 PM

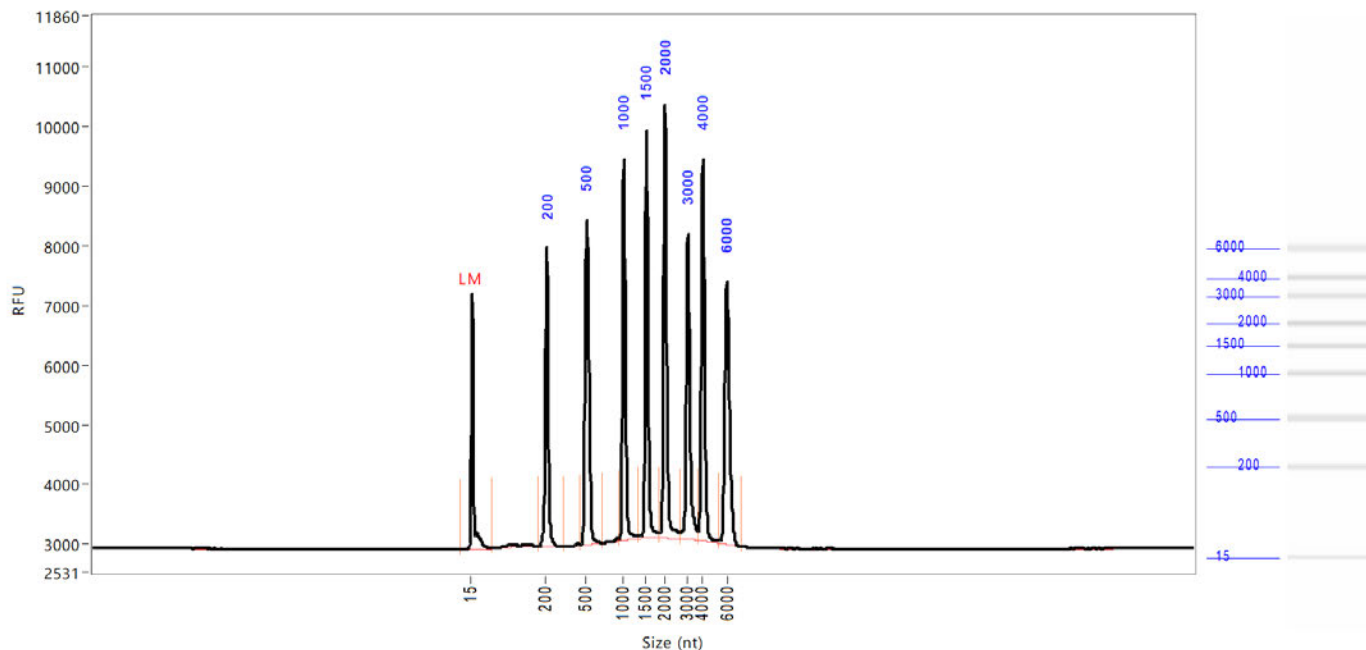


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.7137	5	25	122
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.4257	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0307 ng/ul	7.2 %Total	0.0099 nmole/L	9686 Avg. Size (nt)	11.58 %CV

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Thursday, November 18, 2021 1:39:17 PM



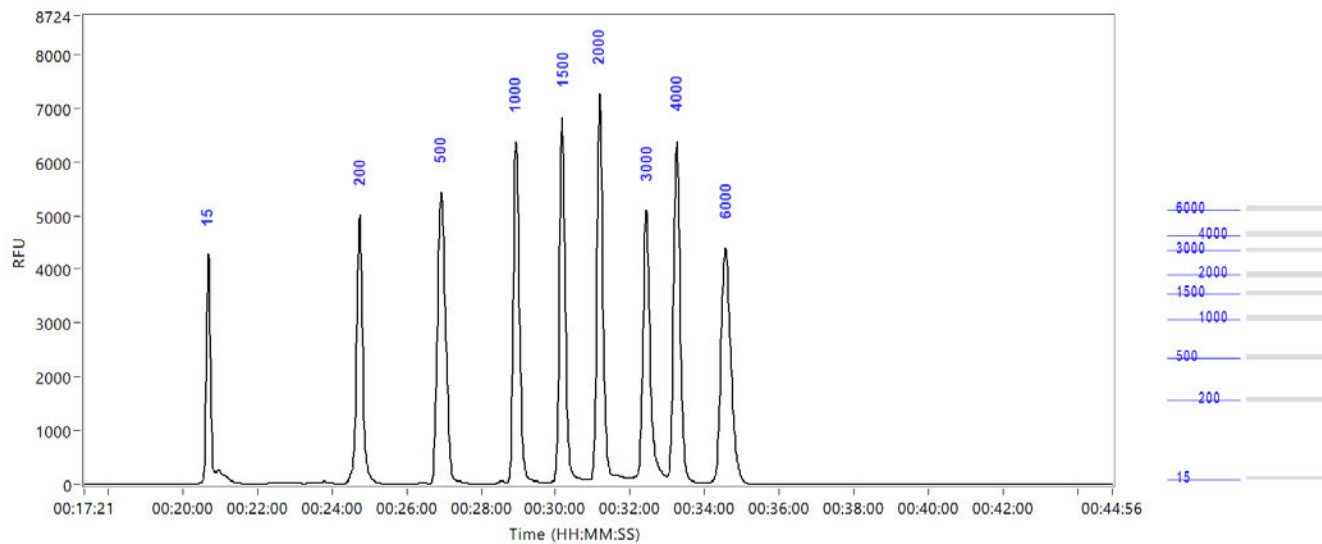
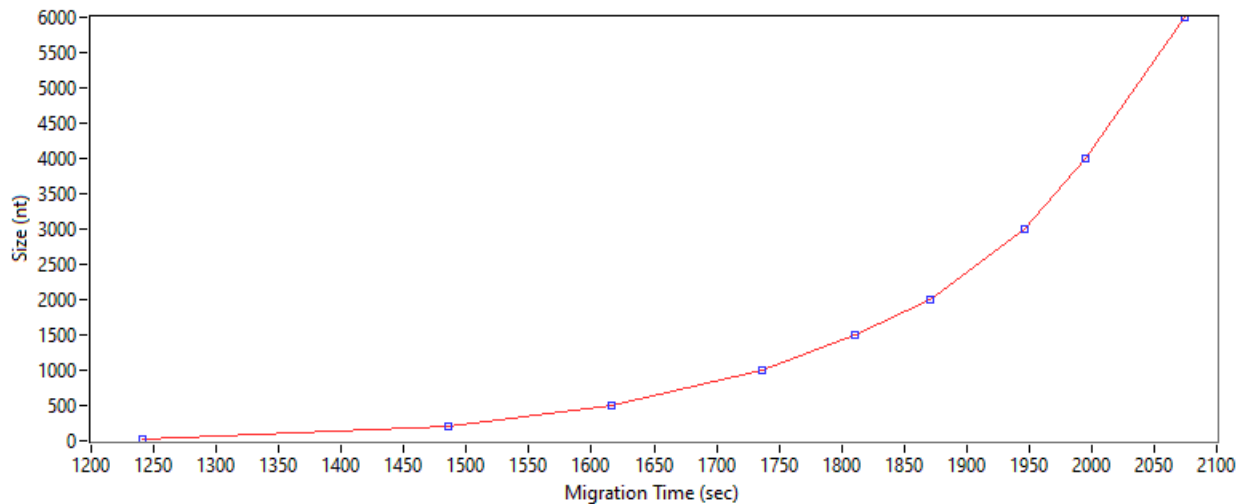
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.7137	0	67	4284
2	200	10.8881	180	328	5014
3	500	14.7793	454	719	5428
4	1000	12.0065	937	1311	6377
5	1500	11.9669	1311	1865	6836
6	2000	13.1046	1865	2636	7268
7	3000	10.6002	2636	3667	5111
8	4000	11.4048	3667	5282	6390
9	6000	11.1244	5282	7191	4406

TIC: 95.8749 ng/uL  
 TIM: 366.7016 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: Y    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Thursday, November 18, 2021 1:39:17 PM  
**Fit type:** Point to point

Calibration curve







Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Appendix 1 - Fragment Analyzer Worksheet - Pfizer COMIRNATY	

### Worksheet for Fragment Analyzer - RNA Integrity

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-26	<b>Analysist</b>	s22
<b>TRIM link to data files</b>	D21-3347281 / D21-3347277	<b>Test Date</b>	18/11/2021

Pipettes & Equipment	
Name	LIMS#
30-300 µL 12 channel pipette	N/A
p10 pipette	32835
p50 pipette	N/A
p100 pipette	32792
p200 pipette	5649
Thermomixer	23660
Thermocycler	N/A
P20	32891

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	N/A
Standard Sensitivity (SS) RNA kit Part 1 stored at 2-8°C	DNF-471-0500	0006609959	10/03/2022
<i>Extra Blank solution</i>	<i>DNF-300-0008</i>	6594431	3/03/2022
Standard Sensitivity (SS) RNA kit Part 2 stored at -20°C (Diluent Marker & Intercalating dye)	Enter text.	Enter text.	Enter a date.
<i>Extra Diluent marker</i>	<i>DNF-369-0004</i>	0006602443	7/04/2023
Standard Sensitivity (SS) RNA kit Part 3 stored at -70°C (RNA Ladder)	DNF-382-U020	0006600148	29/03/2022
Capillary conditioning solution	DNF-475-0100	6598614	22/03/2022
DEPC water	AM9961	2004017	N/A
20% Triton-X100 / 30% Ethanol solution	In house	MC1SEP21-01	1/02/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Intercalating dye	Dnf-600-u030	6575146	25/11/2021
Enter text.	Enter text.	Enter text.	Enter a date.

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT (Drawer B)	18/11/2021	19/11/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT (Drawer M)	18/11/2021	19/11/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT (Drawer 3)	18/11/2021	2/12/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	18/11/2021	2/12/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	18/11/2021	20/11/2021  48 hours
<b>Empty waste tray and waste bottle</b> <b>Reagents can be scaled up if required – this table provides the minimum for a single run.</b> <b>Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</b> <b>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</b>  <b>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</b>			

## 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BF-25	BF-25	BF-25	BF-25	BF-25	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	BF-25	BF-25	BF-25	BF-25	BF-25	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	BF-25	BF-25	BF-25	BF-25	BF-25	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	L

S1-6 = Samples in triplicate (a, b or c),

RM = Reference material

BF-25 = Blank solution provided in kit,

L = RNA ladder

This worksheet assumes the maximum of 6 samples per test. Any samples not included in the test must be crossed off the plate layout, and results table below

System Suitability Criteria – RNA Ladder			
Plate location (wells)	D12		
Parameter	Limits	Results	Comments
RNA ladder profile	Visually comparable to figure 4 of SOP	ok	PASS
All peaks present	15 200 500 1000 1500 2000 3000 4000 6000 nt	ok	PASS
Peak heights	<60000 RFU	ok	PASS
Assay Acceptance Criteria – Reference Material			
Plate location (wells)	A12 B12 C12		
LIMS #	2108002914		
BATCH #	EE8493		
EXPIRY	5/02/2022		
Parameter	Limits	Results	Comments
Profile	Visually comparable to DP electropherogram in SOP	Ok/ok/ok	PASS
Migration time	Approximately comparable to profile in SOP	4078/4052/4104	PASS
Lower marker present	LM peak	Ok/ok/ok	PASS
Peak heights	5000-600000 for 2/3 replicates	15553/14183/13671	PASS
No negative peaks or baseline shifts	No significant peaks/shifts	Ok/ok/ok	PASS
Reference Material Dilutions / Calculation / Notes			
thaw date: 08/11/21 270ng/uL = 20 uL of 530 ng/uL master stock + 19 uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution  The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919			

Sample 1 Details	
Plate location (wells)	A11 B11 C11
LIMS #	2111004115
BATCH #	FL5333
EXPIRY	28/02/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4078/4052/ 4078	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	16700/18546/ 17252	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>opened for the first time 15/11/21, stored at 2-8C                      270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water                      90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919</p>

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 2 Details	
Plate location (wells)	A10 B10 C10
LIMS #	2111004218
BATCH #	FK0738
EXPIRY	30/06/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4027/ 4078/ 4052	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	20552/ 20216/ 18272	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>opened for the first time 18/11/21, stored at 2-8C                      270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water                      90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919 opened for the first time</p>

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 3 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	PASS
Lower marker	LM must be present	Enter text.	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
Thaw date – 25/10/2021 – stored cell culture fridge , opened for the first time 25/10/21, stored at 2-8C 60 ng/uL = 20 uL of 200 ng/uL DP + 40 uL %Tx100 solution/30% ethanol  The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919

Sample Results	
Choose an item.	
Analysist	s22
Checked by	Enter text.
Sample 4 Details	
Plate location (wells)	A8 B8 C8
LIMS #	2110003932

<b>BATCH #</b>	s22
<b>EXPIRY</b>	5/05/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	3981/3981/3942	Choose an item.
Lower marker	LM must be present	Ok/ok/ok	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	13683/11870/13713	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
<b>Analysist</b>	Enter text.
<b>Checked by</b>	Enter text.

Sample 5 Details	
<b>Plate location (wells)</b>	Choose an item.
<b>LIMS #</b>	Click or tap here to enter text.
<b>BATCH #</b>	Click or tap here to enter text.
<b>EXPIRY</b>	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.

Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.
Sample 6 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.

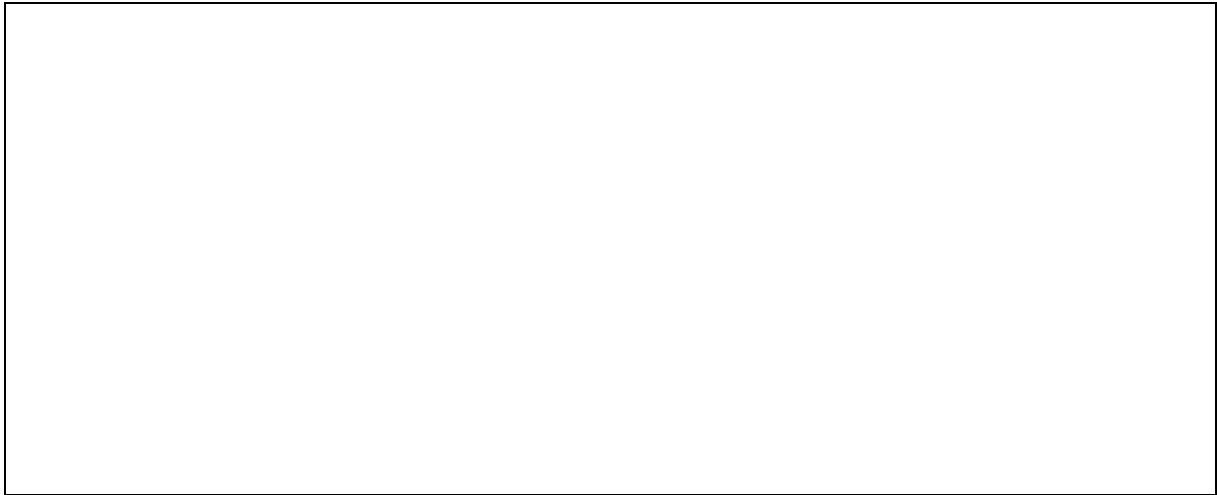


<b>% Late Migrating Species</b>		Enter text.	Enter text.	Enter text.	
---------------------------------	--	-------------	-------------	-------------	--

<b>Sample Dilutions / Calculation / Notes</b>
Enter text.

<b>Sample Results</b>	
<b>Choose an item.</b>	
<b>Analysist</b>	
<b>Checked by</b>	Enter text.

<b>Notes</b>
Enter text.





<b>Owner:</b> s22	<b>Number:</b> Bio-BEE-Form-42
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 2/07/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 18Nov2021

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899LRW Expiry: 18 March 2022Other Reagent: Pyrospense Batch# 0000904583  
November 2021Expiry: 29 June 2022 Use By: 19

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Pfizer Covid Vaccine</u>	<u>FL5333</u>	<u>28 February 2022</u>	<u>2111004115-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
<u>n/a</u>	s47	<u>2500</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of vaccine</u>	<u>975uL</u>	<u>5uL Pyrospense</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrospense</u>
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>1.43</u>	s47	<u>1.32</u>	<u>115</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

**Notes:**

**Checked** s22 18Nov2021

## Instrument controller software run summary:

**Filename and data path:** \\central.health\dfsuserenv\Users\User\_01\CONTEM\Documents\2 - Fragment Analyzer Data\2021 11 18 - pfizer\2021 11 18 13H 13M.raw

**Created:** Thursday, November 18, 2021 1:39:17 PM

**Number of capillaries:** 12

**Array serial number:** 022621-27SFS

**Effect length:** cm

**Array usage count:**

**Instrument type:** 5200 Fragment Analyzer

**Instrument controller software version:** 3.1.0.12

**Device serial number:** MY2105AB19

### Method Information

**Method name:** Separation Method

**Gel prime:** No

**Full conditioning:** No

**Gel prime to buffer:** No

**Gel selection:** Gel 1

**Perform prerun:** No

**Rinse:** No

**Marker 1:** No

**Rinse:** No

**Sample injection:** No

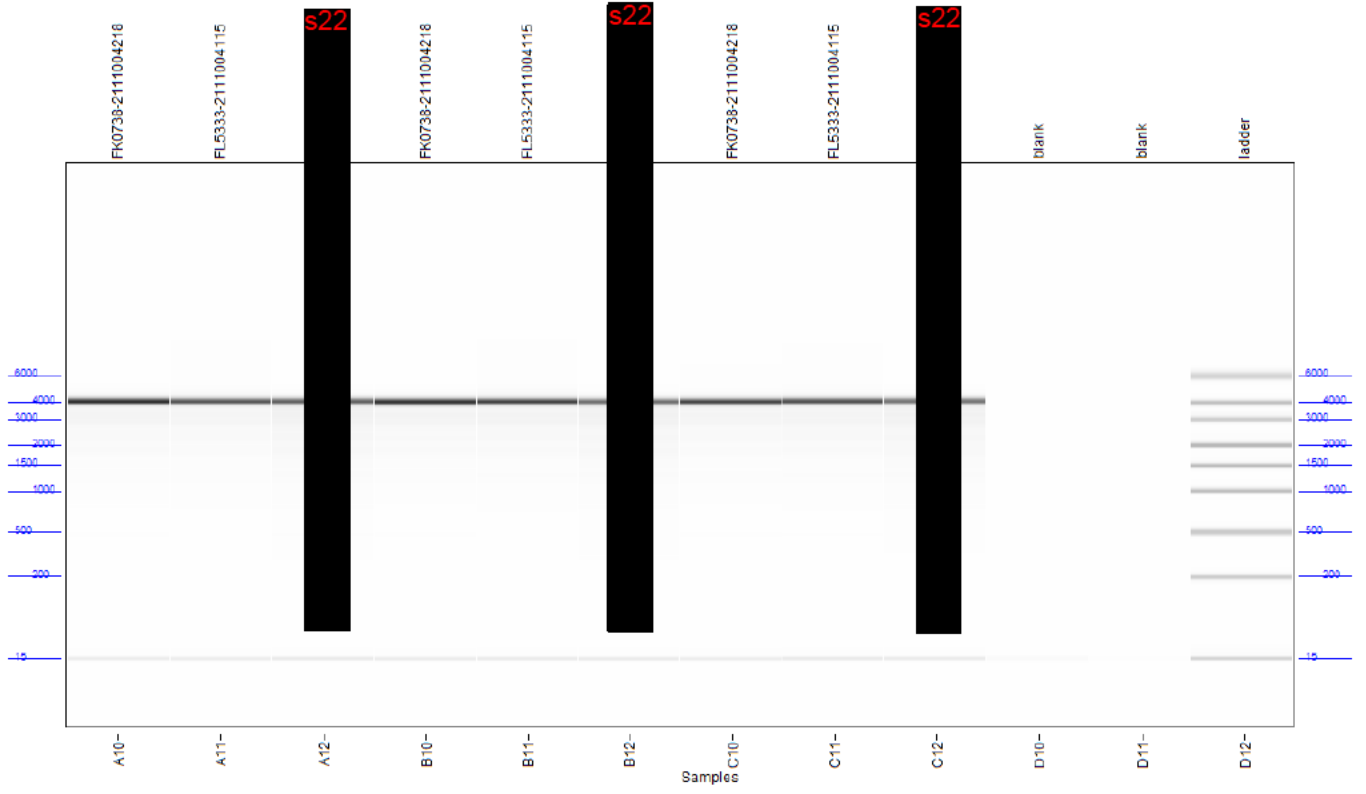
**Separation:** No

**Tray name:**

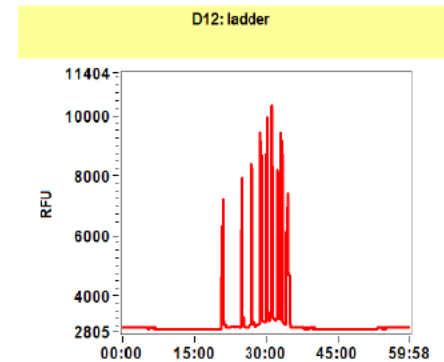
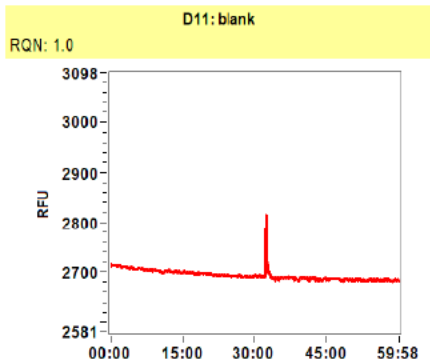
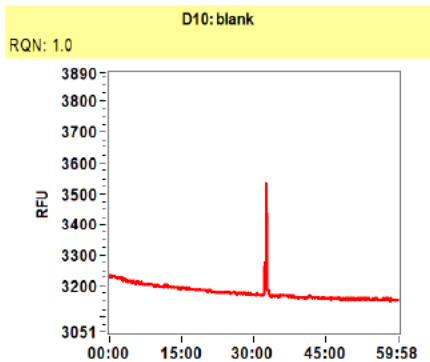
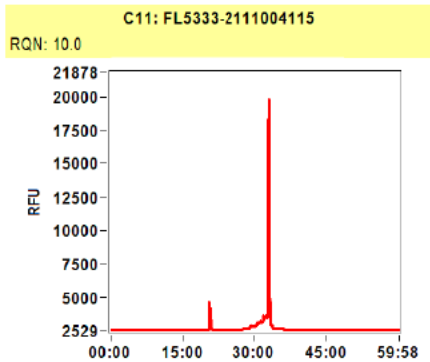
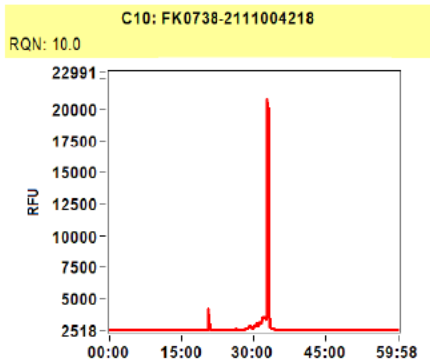
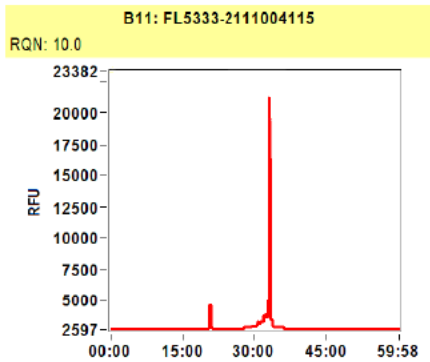
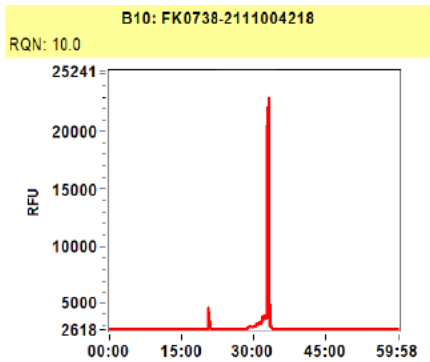
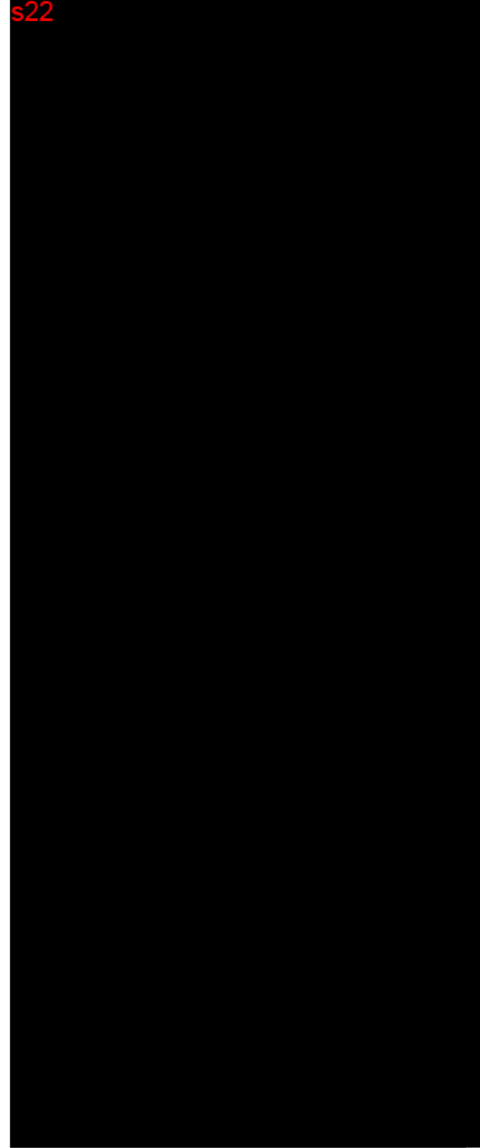
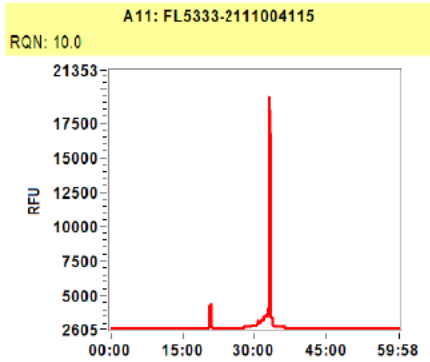
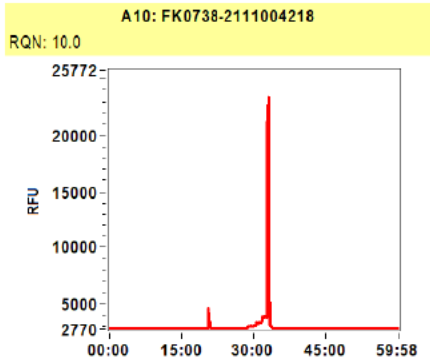
**Analysis mode:** RNA (Eukaryotic)

### Notes

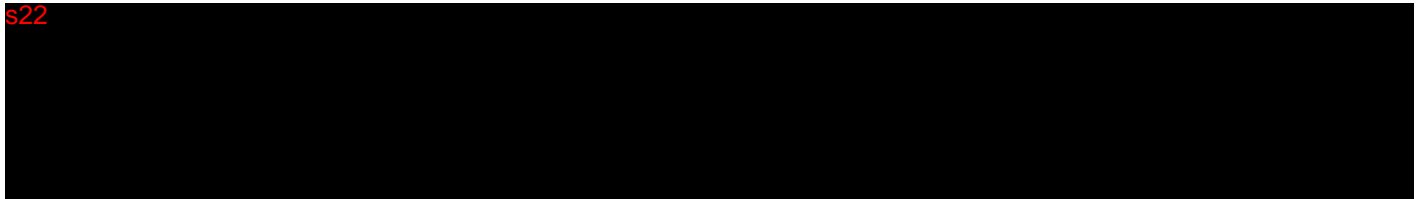
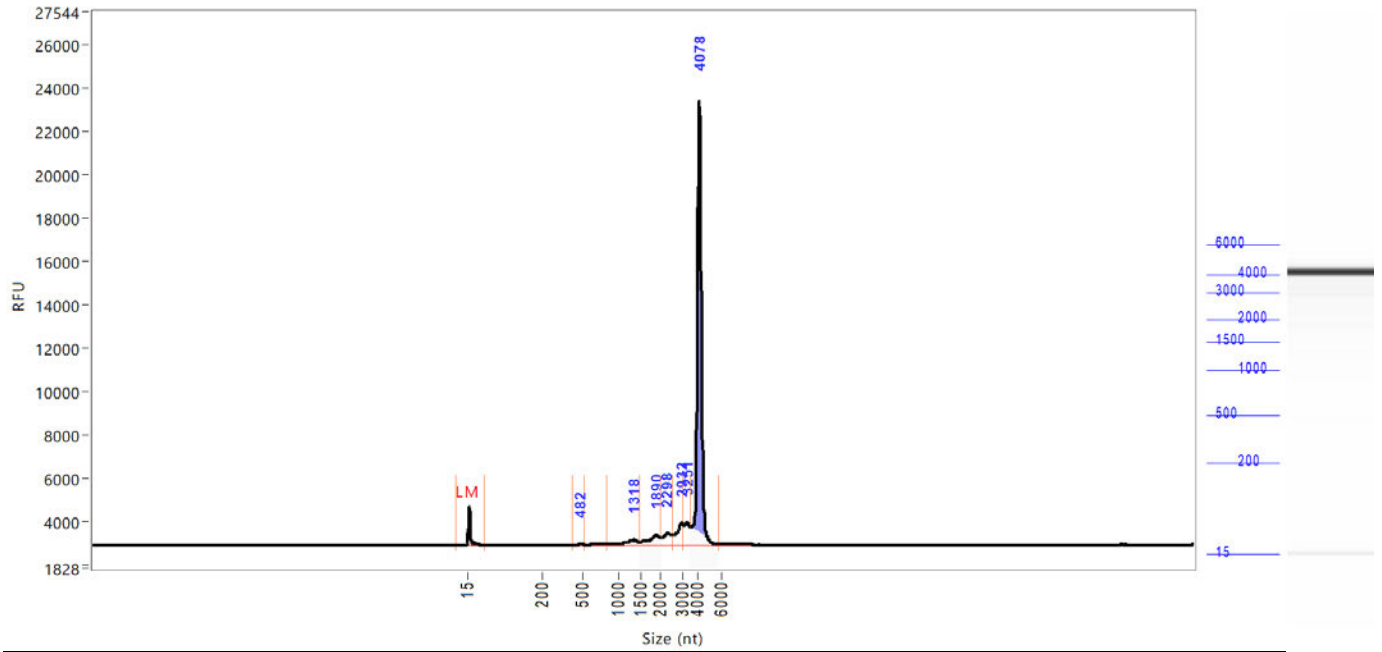
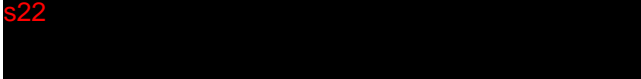
### Gel Image



s22

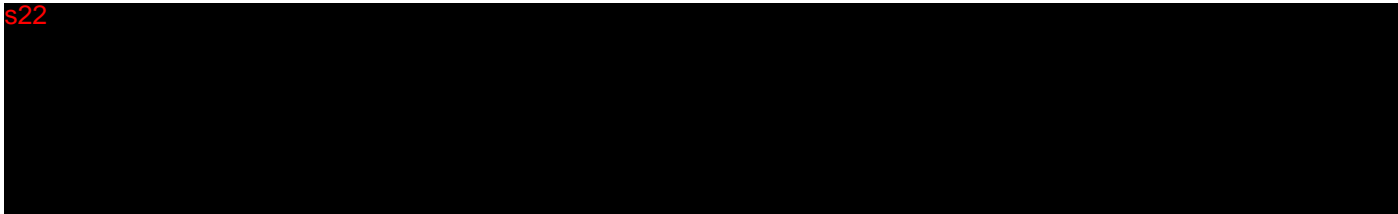
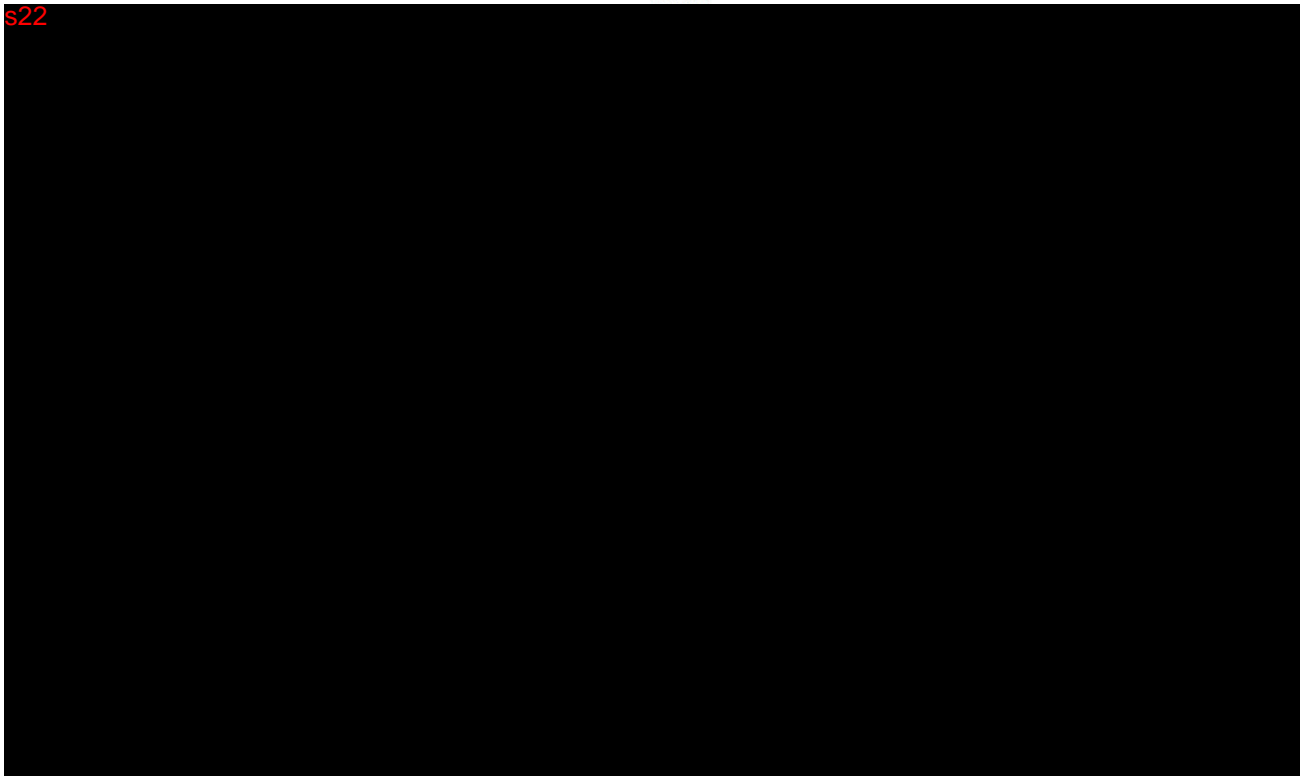
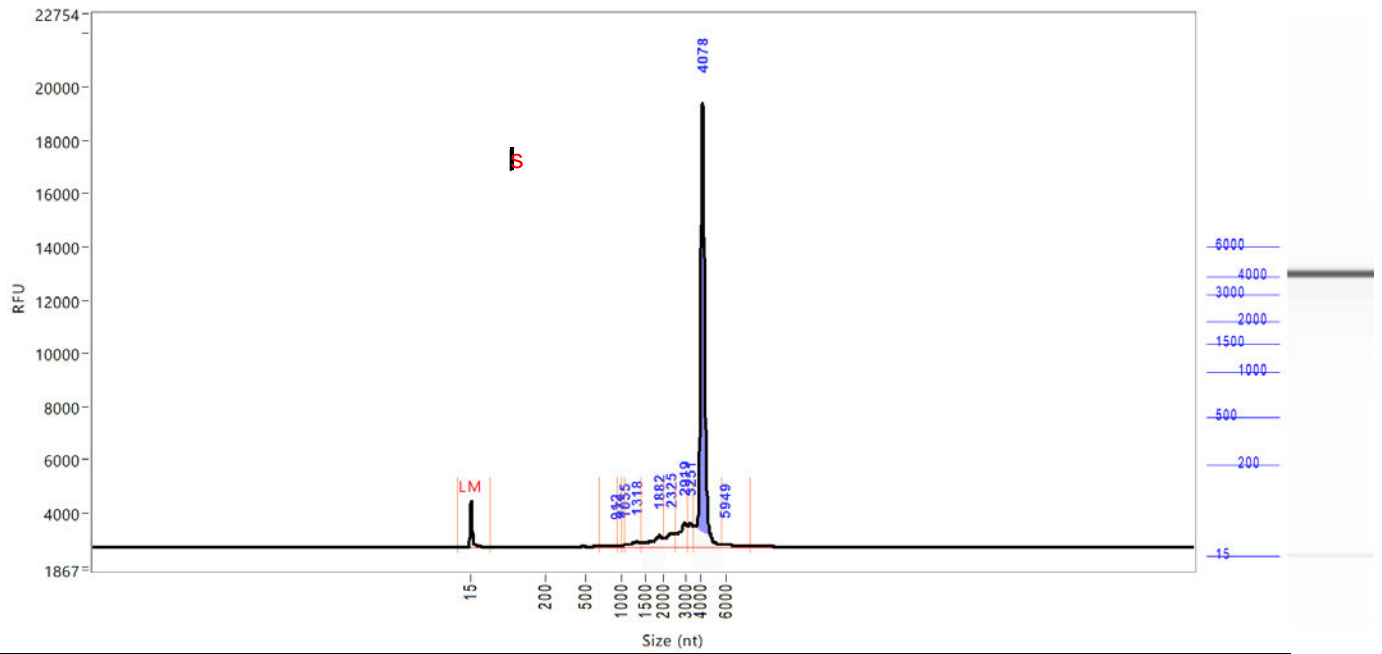


Sample: EK0738-2111004218





Sample: FI 5333-2111004115



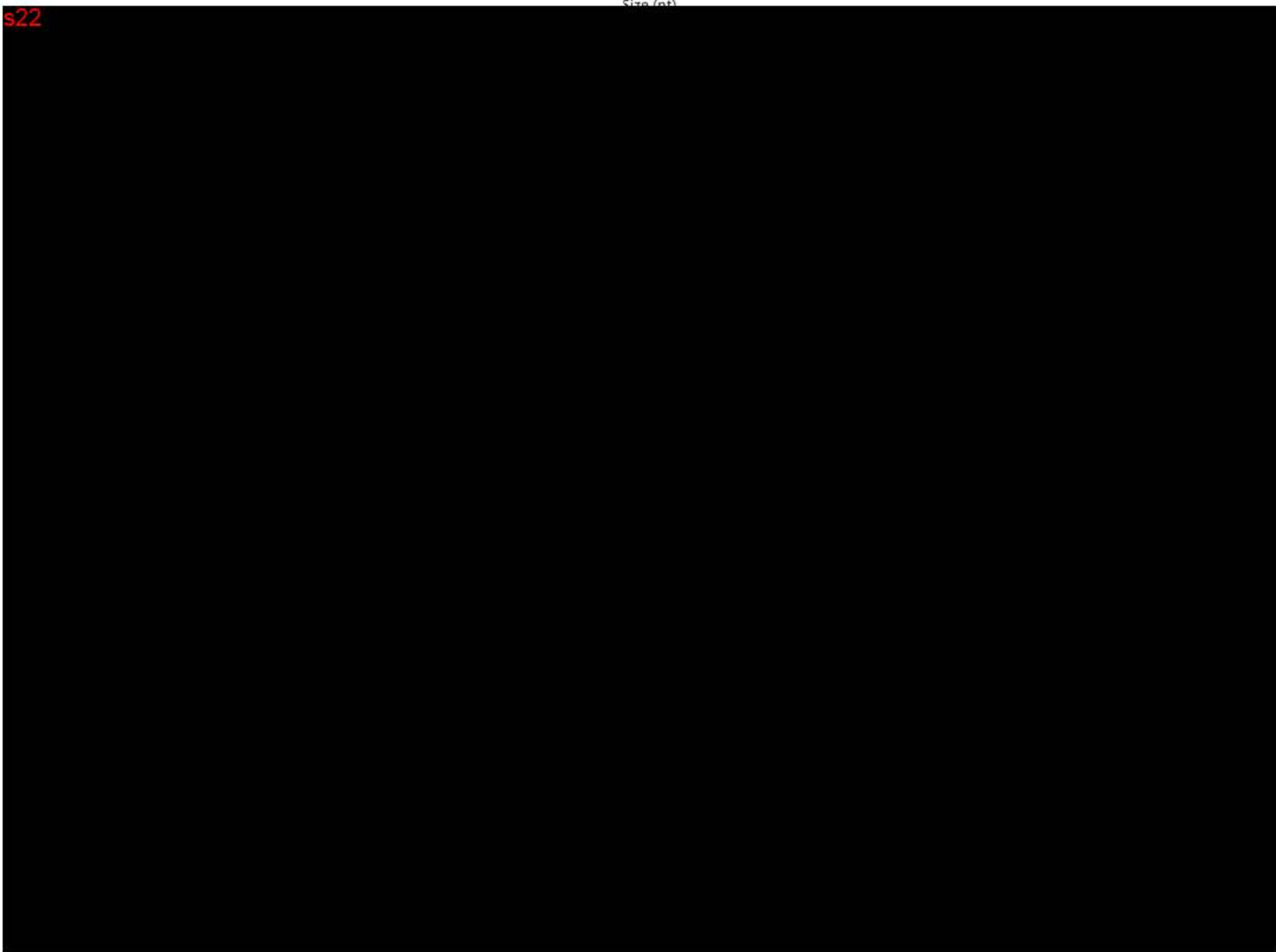
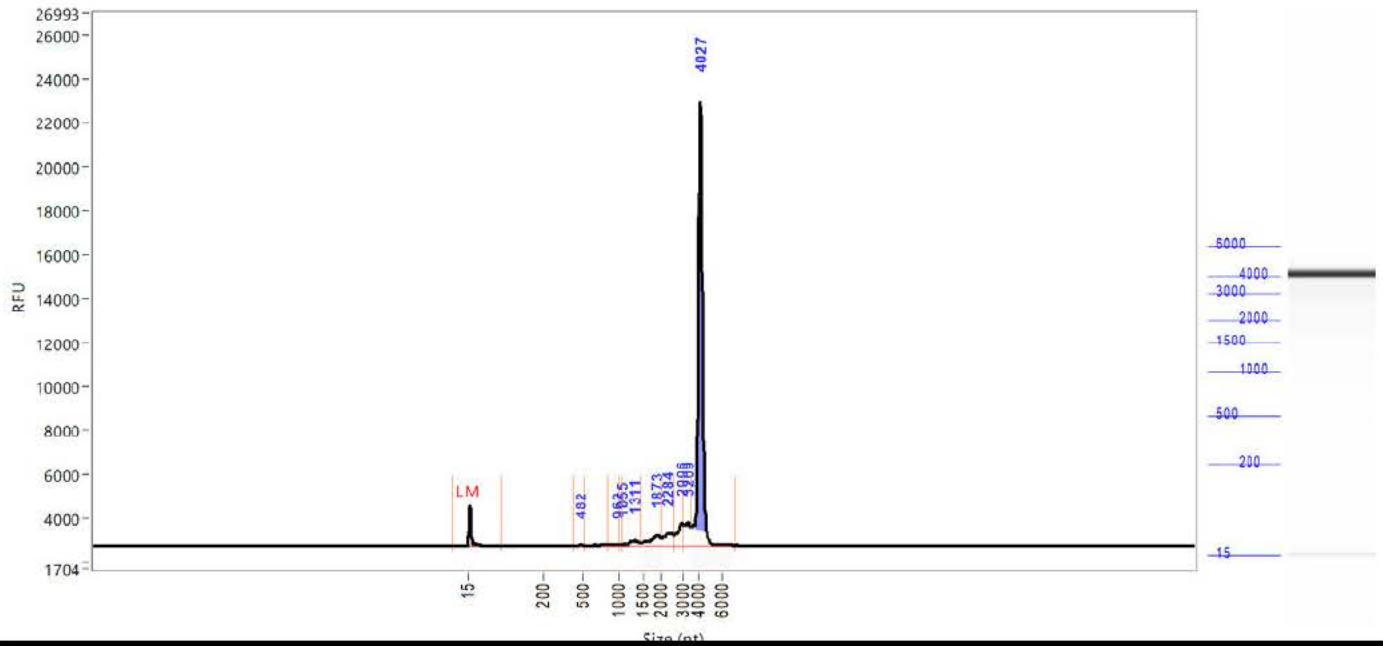
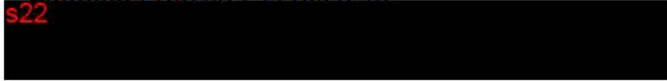
s22



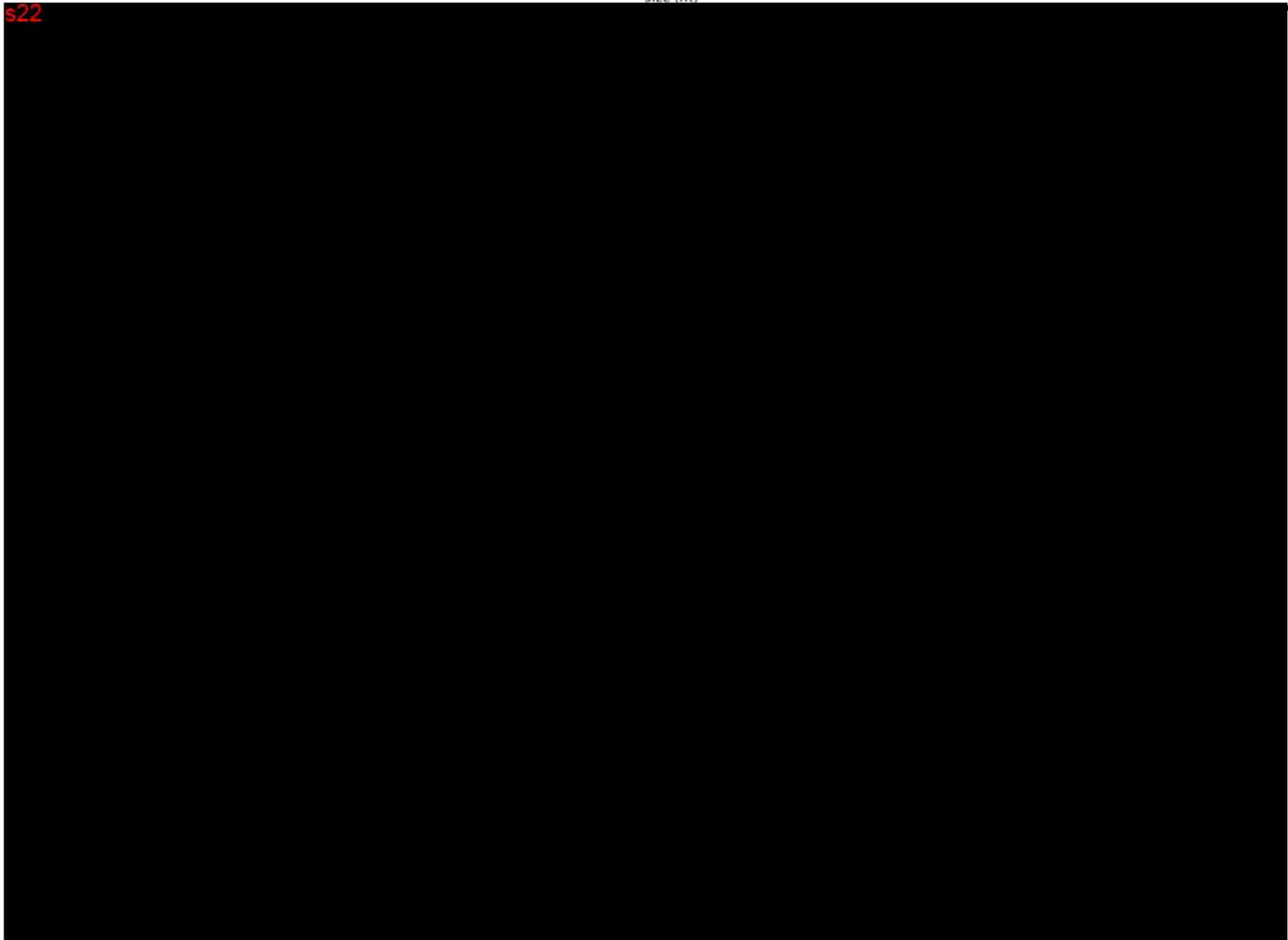
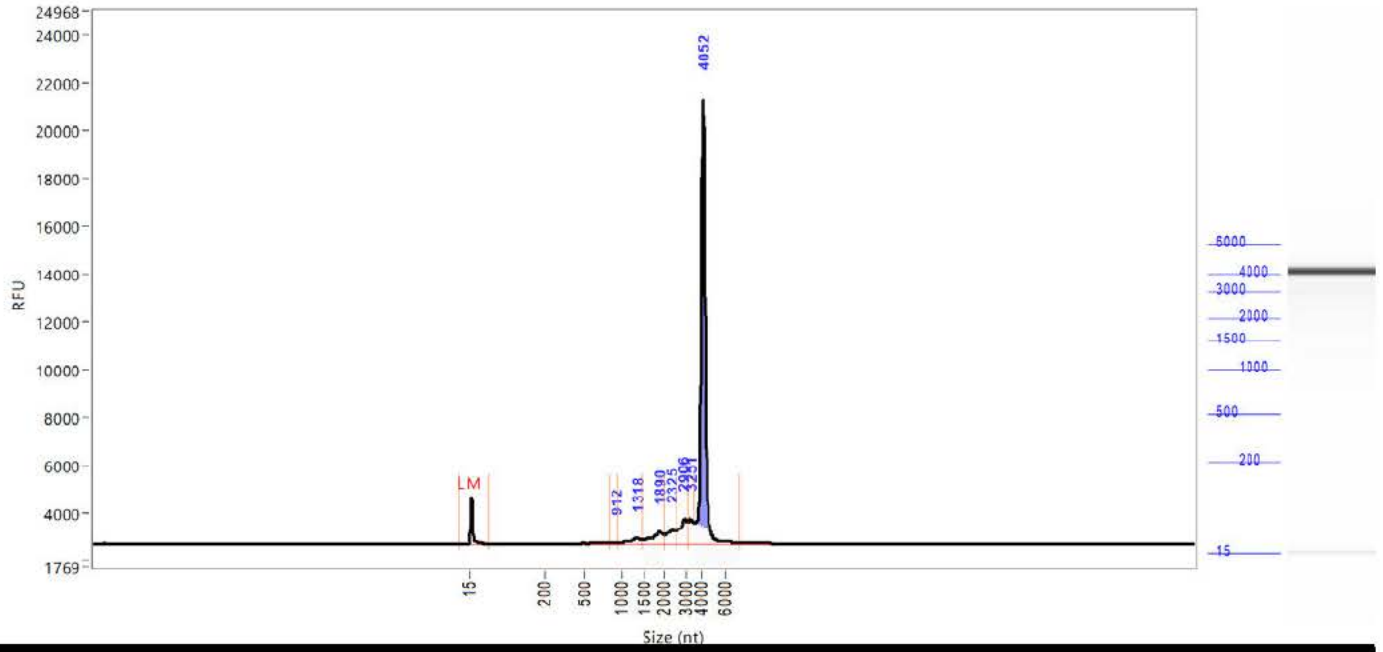
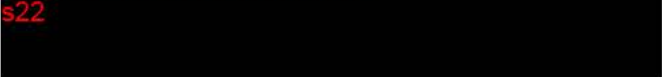
s22



Sample: EK0738-2111004218



Sample: FI 5333-2111004115



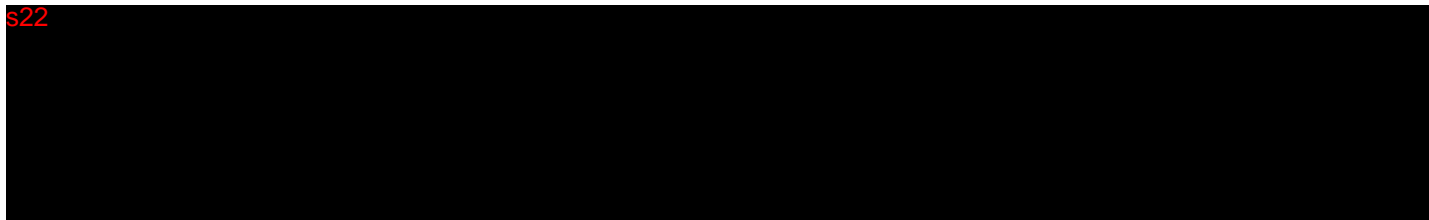
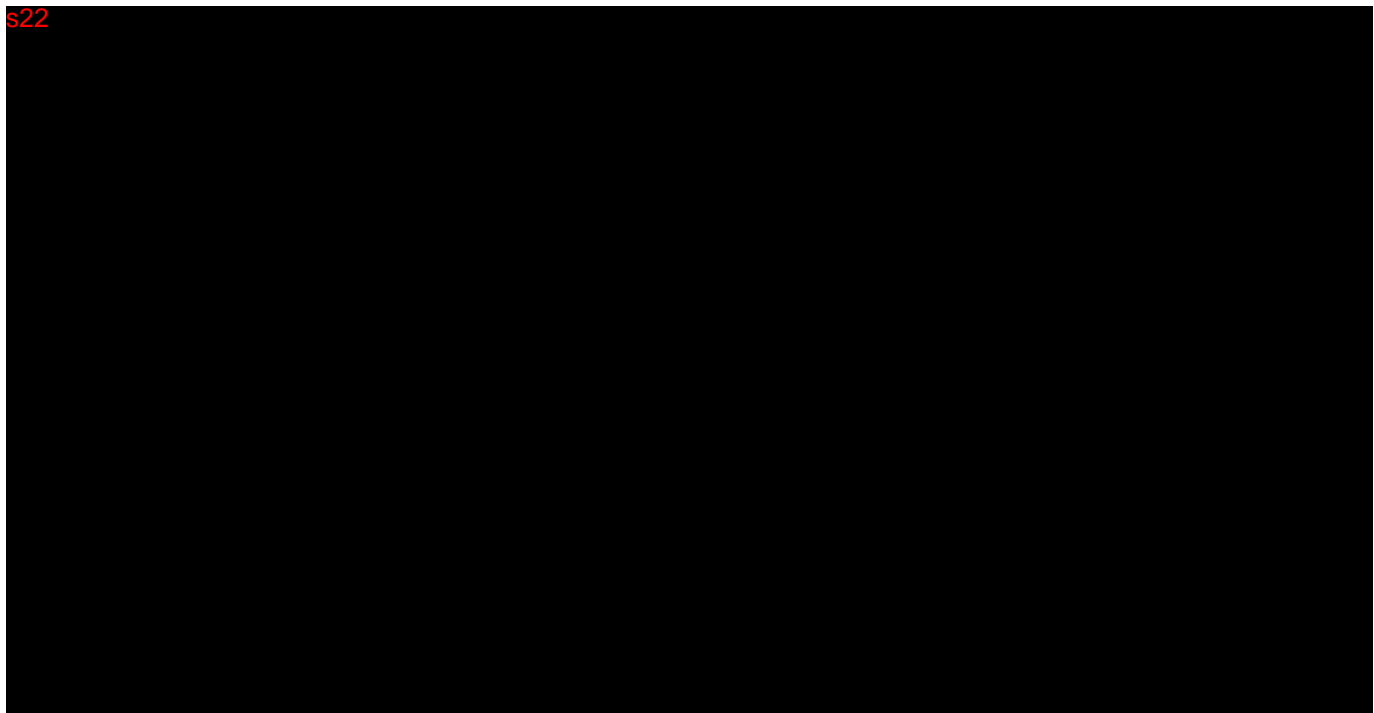
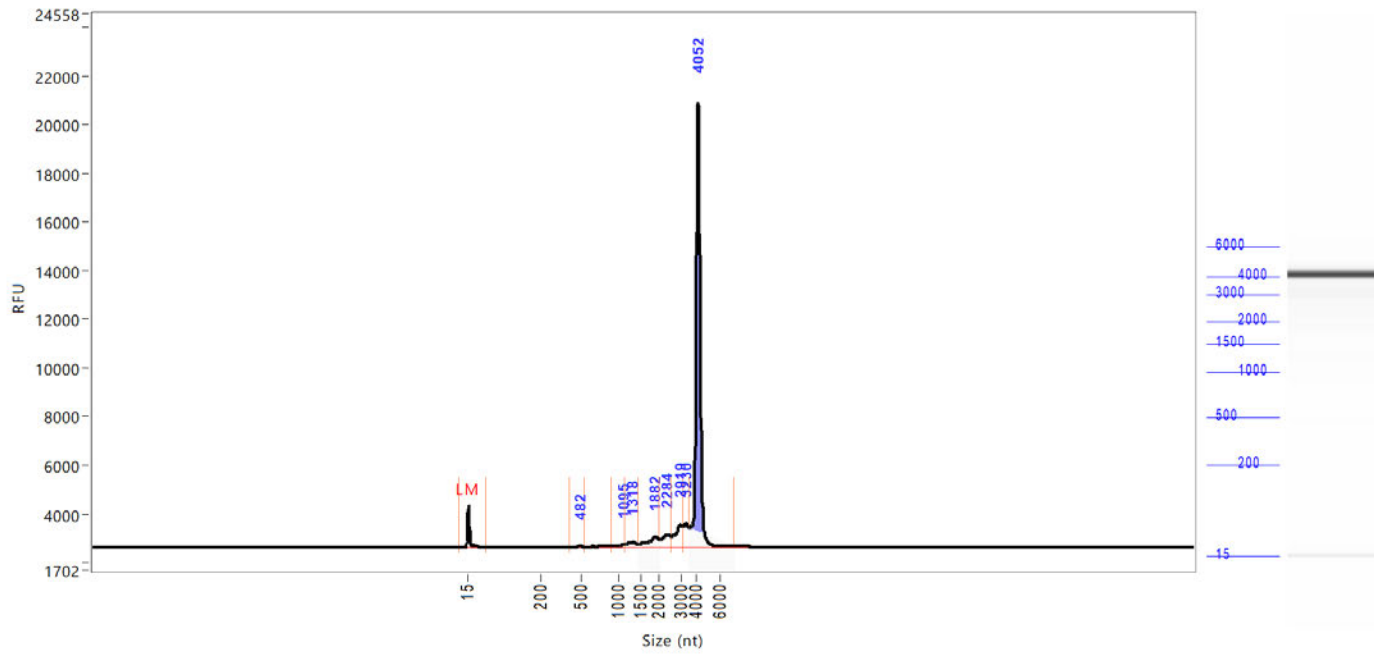
s22



s22

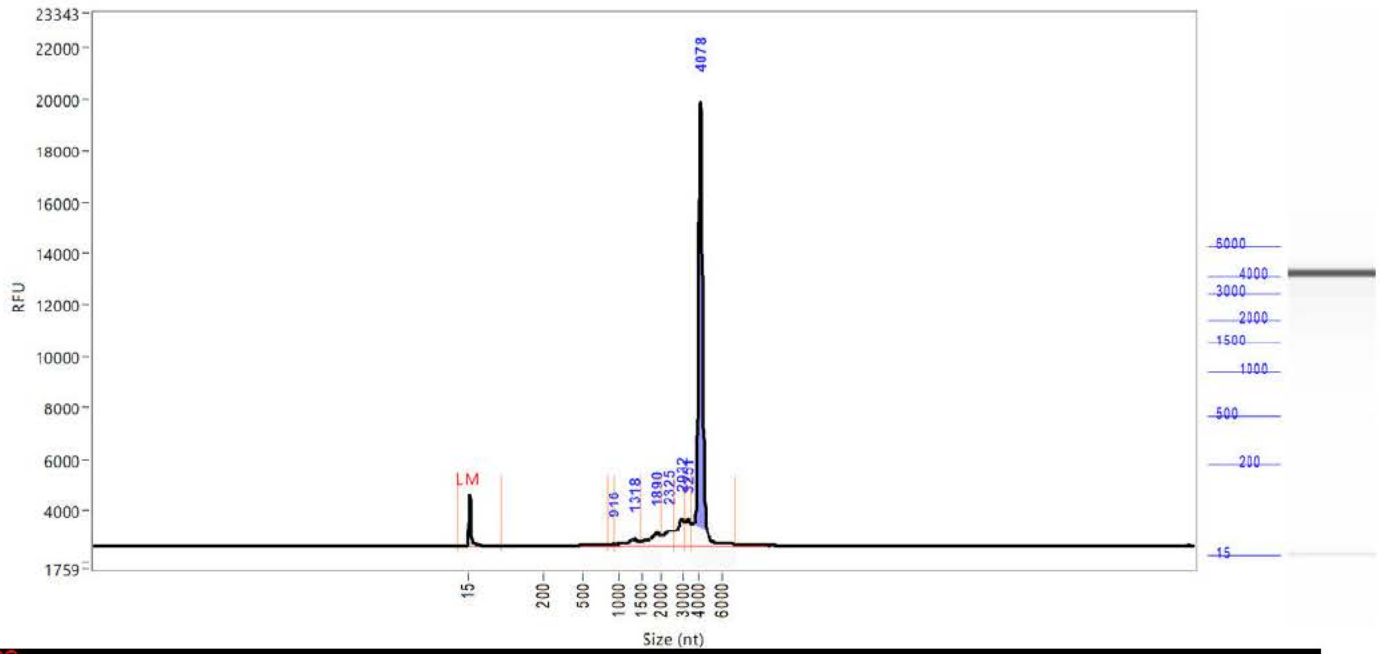


Sample: FK0738-2111004218



Sample: FI 5333-2111004115

s22



s22

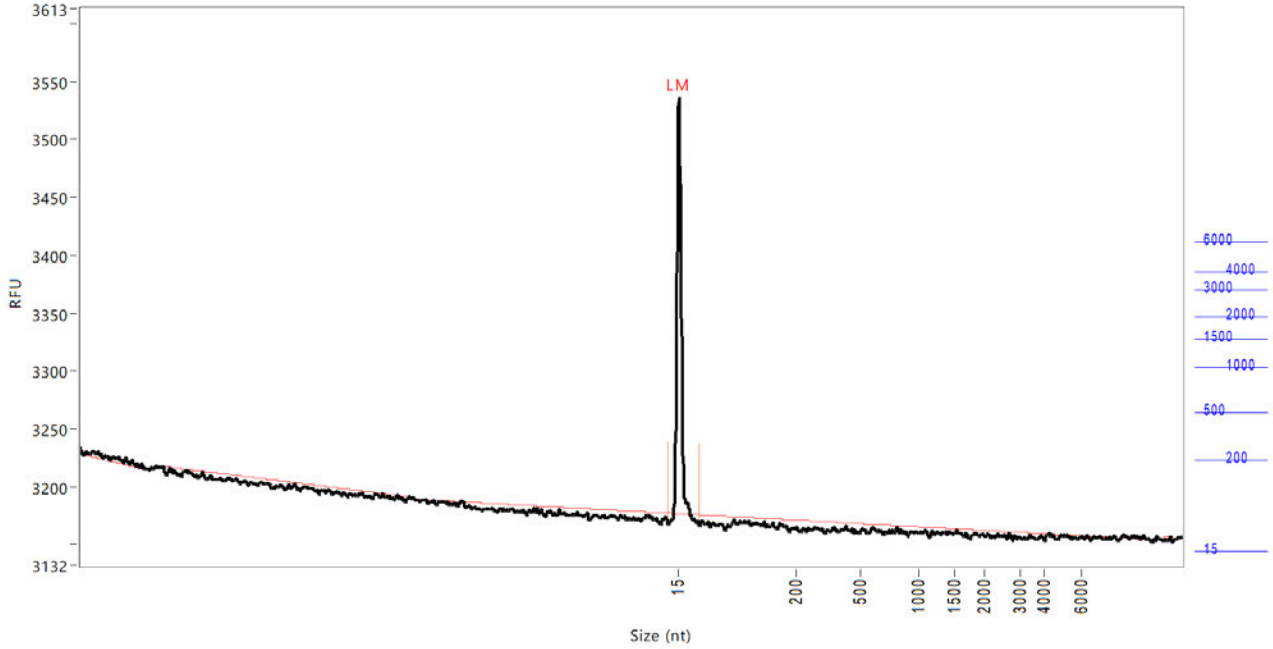
s22

S22





**Sample:** blank  
**Well location:** D10  
**Created:** Thursday, November 18, 2021 1:39:17 PM

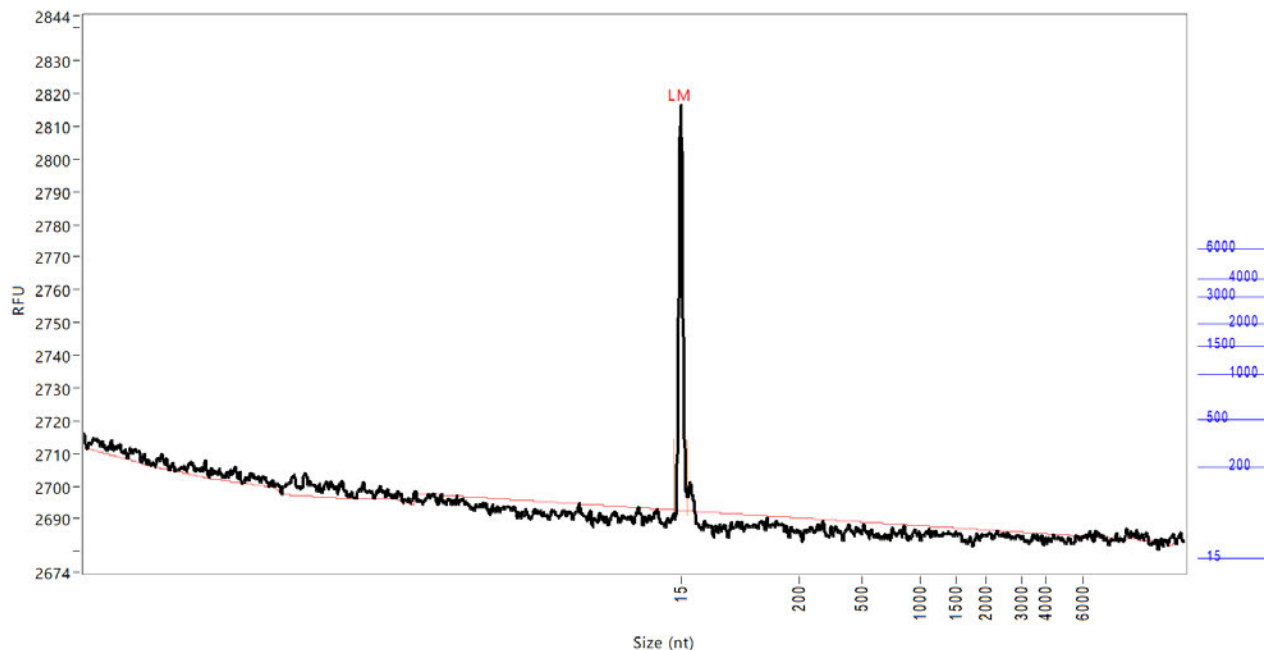


Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.0446	0	48	358
TIC:		0.0000	ng/uL		
TIM:		0.0000	nmole/L		
Total concentration:		0.0010	ng/uL		
28s/18s:		0.0			
RQN		1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/uL	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0010 ng/uL	100.0 %Total	0.0003 nmole/L	9147 Avg. Size (nt)	7.07 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 0.5000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** blank  
**Well location:** D11  
**Created:** Thursday, November 18, 2021 1:39:17 PM

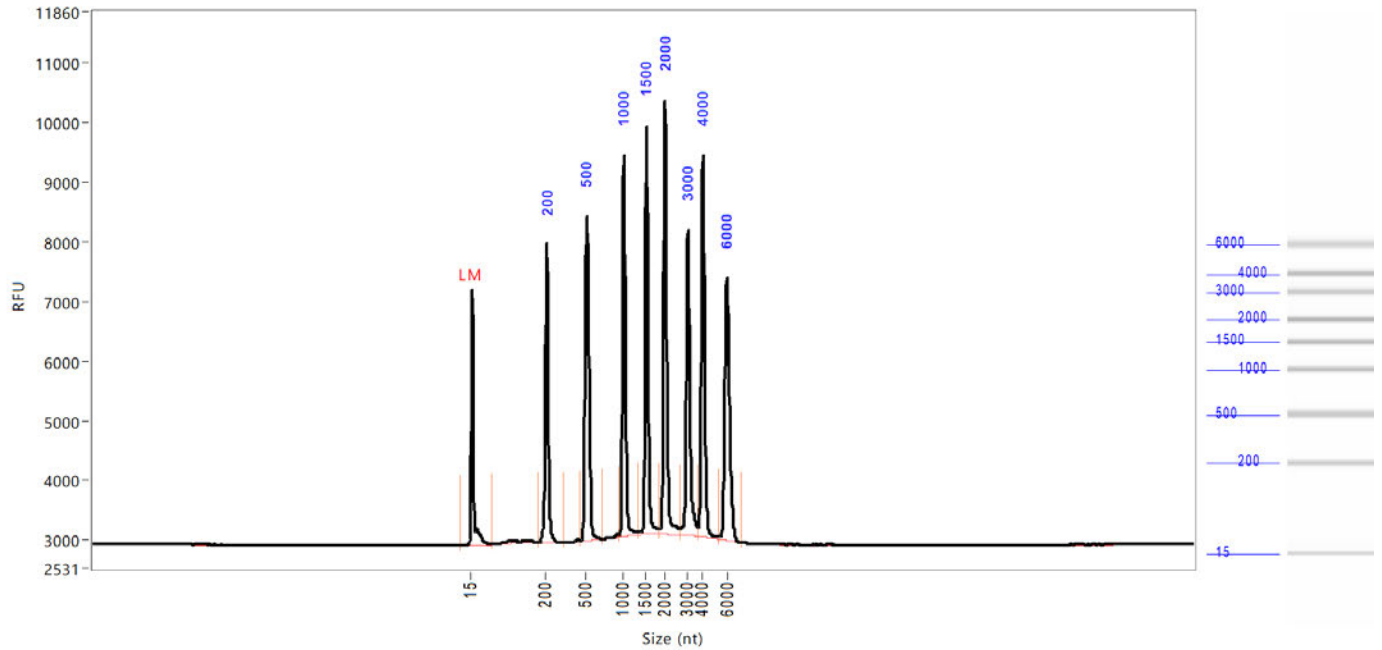


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.0446	5	25	122
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0266	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0019 ng/ul	7.2 %Total	0.0006 nmole/L	9686 Avg. Size (nt)	11.58 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
Manual baseline start (min): 18    Manual baseline end (min): 59  
Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
Quantification using: Ladder    Final concentration (ng/uL): 0.5000    Dilution factor: 12.0  
Minimum RFU for data processing: 2

**Sample:** ladder  
**Well location:** D12  
**Created:** Thursday, November 18, 2021 1:39:17 PM



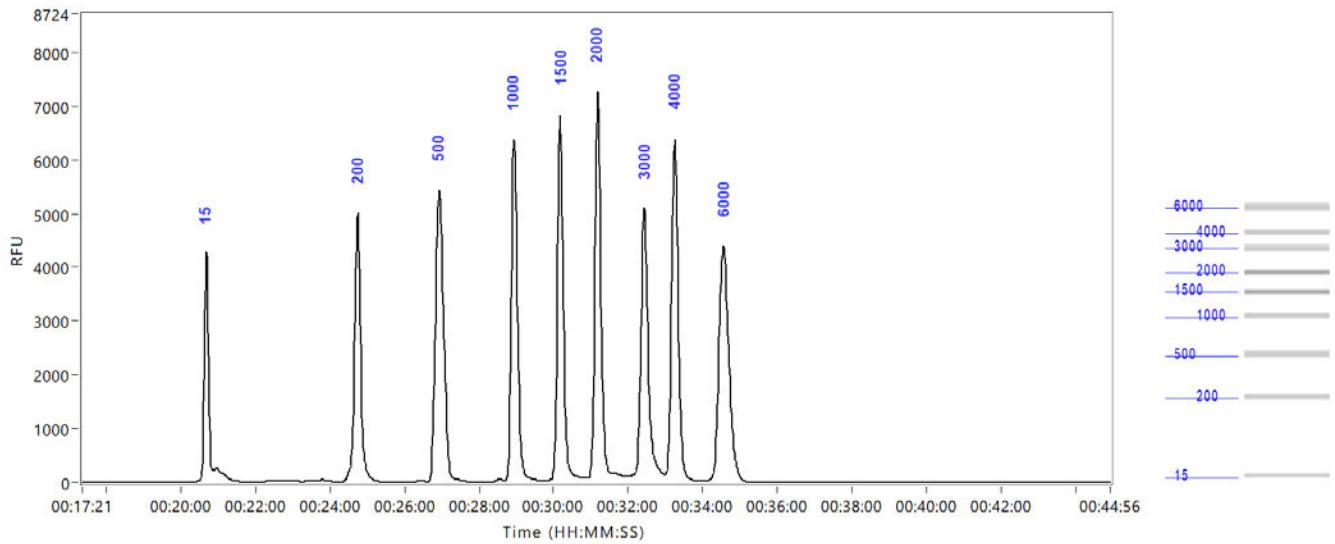
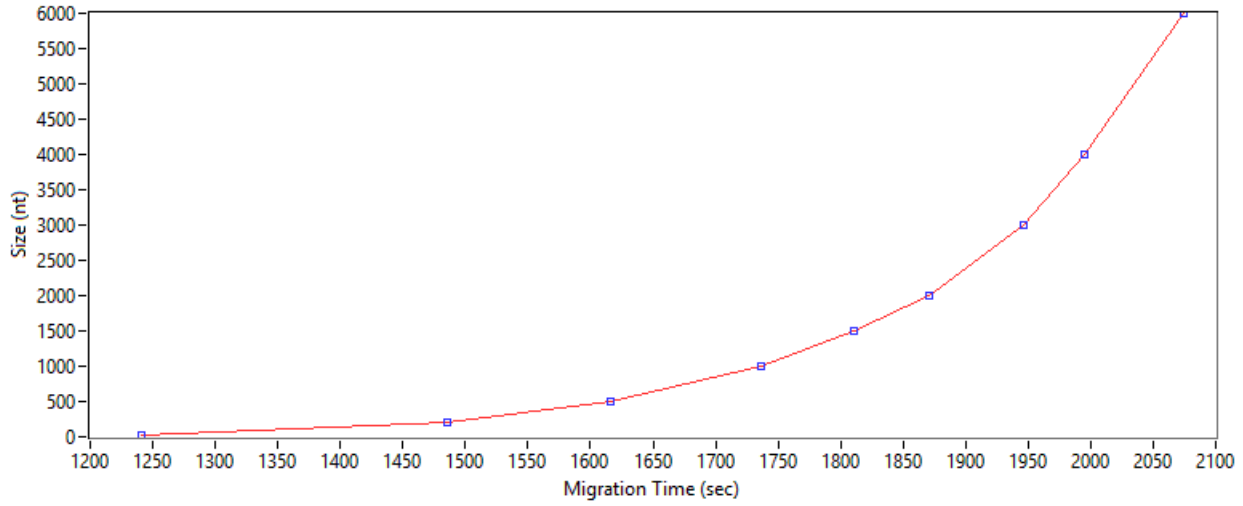
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.5000	0	67	4284
2	200	7.6275	180	328	5014
3	500	10.3534	454	719	5428
4	1000	8.4110	937	1311	6377
5	1500	8.3832	1311	1865	6836
6	2000	9.1802	1865	2636	7268
7	3000	7.4258	2636	3667	5111
8	4000	7.9895	3667	5282	6390
9	6000	7.7931	5282	7191	4406

TIC: 67.1636 ng/uL  
 TIM: 256.8871 nmole/L  
 Total concentration: 67.7209 ng/uL

Sample peak width (sec): 6      Sample min peak height: 200      Sample baseline V to V?: Y      Sample baseline V to V points: 3  
 Sample filter: Binomial      Number of points for filter: 9      Sample start region (min): 0      Sample end region (min): 60  
 Marker peak width (sec): 6      Marker min peak height: 200      Marker baseline V to V?: N      Marker baseline V to V points: 3  
 Lower marker selection: First peak > 200 RFU      Upper marker selection: Last peak > 200 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Lower Marker      Final concentration (ng/uL): 0.5000      Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** ladder  
**Well location:** D12  
**Created:** Thursday, November 18, 2021 1:39:17 PM  
**Fit type:** Point to point

Calibration curve



## Data from Smear Analysis Table

1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate  
 2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the avg smear sets.  
 3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
B9	B9	RM-Moderna	3700 nt to 4800 nt	72.4229	84.9	56.2473	4017	4.01
B9	B9	RM-Moderna	4800 nt to 13000 nt	0.5515	0.6	0.2846	6046	18.26
B10	B10	FK0738-2111004						
B10	B10	FK0738-2111004						
B11	B11	FL5333-2111004						
B11	B11	FL5333-2111004						
C9	C9	RM-Moderna	3700 nt to 4800 nt	70.9436	84.2	55.0723	4019	4.01
C9	C9	RM-Moderna	4800 nt to 13000 nt	0.5159	0.6	0.267	6028	18.62
C10	C10	FK0738-2111004						
C10	C10	FK0738-2111004						
C11	C11	FL5333-2111004						
C11	C11	FL5333-2111004						
D4	D4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D6	D6	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3833	2.48
D6	D6	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	10814	0.14
D7	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	4800 nt to 13000 nt	0.0011	25.5	0.0003	11070	4.54
D8	D8	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D8	D8	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
D9	D9	Blank	3700 nt to 4800 nt	0.006	0.8	0.005	3733	0.58
D9	D9	Blank	4800 nt to 13000 nt	0.0154	2.1	0.0047	10252	12.09
D10	D10	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D10	D10	Blank	4800 nt to 13000 nt	0.0162	100	0.0055	9147	7.07
D11	D11	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D11	D11	Blank	4800 nt to 13000 nt	0.0307	7.2	0.0099	9686	11.58
D12	D12	Ladder						

Written By [REDACTED]  
Authorised [REDACTED]

Date Validated 10/06/2021  
Validation Due 10/06/2022

Revision no. 1  
LIMS number 33325

Validation Status **Validation OVERDUE**  
Analyst [REDACTED]  
Assay Date 17/11/2021

Pass/Fail Parameters

minimum	cut off	maximum
[REDACTED]	[REDACTED]	[REDACTED]
result >>	[REDACTED]	[REDACTED]

										% INTEGRITY SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
1	A9	RM-Moderna	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	RM-Moderna	[REDACTED]	[REDACTED]	[REDACTED]	PASS	
2	B9	RM-Moderna	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
3	C9	RM-Moderna	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
1	A10	FK0738-2111004218	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	FK0738-2111004218	[REDACTED]	[REDACTED]	[REDACTED]	PASS	
2	B10	FK0738-2111004218	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
3	C10	FK0738-2111004218	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
1	A11	FL5333-2111004115	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	FL5333-2111004115	[REDACTED]	[REDACTED]	[REDACTED]	PASS	
2	B11	FL5333-2111004115	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
3	C11	FL5333-2111004115	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
1	D4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
3	D6	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3833	2.48	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
1	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
2	D8	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
3	D9	Blank	3700 nt to 4800 nt	0.006	0.8	0.005	3733	0.58	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	

										% LATE MIGRATING SPECIES SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
1	A9	RM-Moderna	4800 nt to 13000 nt	1.9444	2.1	0.7657	7922	27.86	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]	
2	B9	RM-Moderna	4800 nt to 13000 nt	0.5515	0.6	0.2846	6046	18.26	RM-Moderna	1.10	0.87	78.73	[REDACTED]	

3	C9	RM-Moderna	[REDACTED]							[REDACTED]			
1	A10	FK0738-2111004218	[REDACTED]							FK0738-2111004218			
2	B10	FK0738-2111004218	[REDACTED]							[REDACTED]			
3	C10	FK0738-2111004218	[REDACTED]							[REDACTED]			
1	A11	FL5333-2111004115	[REDACTED]							FL5333-2111004115			
2	B11	FL5333-2111004115	[REDACTED]							[REDACTED]			
3	C11	FL5333-2111004115	[REDACTED]							[REDACTED]			
[REDACTED]													
1	D4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
2	D5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
3	D6	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	10814	0.14	Blank	0.00	0.00	#DIV/0!	
1	D7	Blank	4800 nt to 13000 nt	0.0011	25.5	0.0003	11070	4.54	Blank	13.80	16.55	119.90	
2	D8	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN	Blank	13.80	16.55	119.90	
3	D9	Blank	4800 nt to 13000 nt	0.0154	2.1	0.0047	10252	12.09	Blank	13.80	16.55	119.90	

This tab is only to be used if a replace needs to be excluded from the analysis.

Enter raw data directly into this table. All averages/ pass fail will be calculated automatically using the pass/fail parameters entered in the Calculations tab.

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS	
									Sample ID	Average	stdev	%CV		
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

Pass/Fail Parameters	
s47	
result >>	s47

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
									Sample ID	Average	stdev	%CV	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	









<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Appendix 1 - Fragment Analyzer Worksheet - Pfizer COMIRNATY	

### Worksheet for Fragment Analyzer - RNA Integrity

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-26	<b>Analysist</b>	s22
<b>TRIM link to data files</b>	D21-3347281 / D21-3347277	<b>Test Date</b>	18/11/2021

Pipettes & Equipment	
Name	LIMS#
30-300 µL 12 channel pipette	N/A
p10 pipette	32835
p50 pipette	N/A
p100 pipette	32792
p200 pipette	5649
Thermomixer	23660
Thermocycler	N/A
P20	32891

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	N/A
Standard Sensitivity (SS) RNA kit Part 1 stored at 2-8°C	DNF-471-0500	0006609959	10/03/2022
<i>Extra Blank solution</i>	<i>DNF-300-0008</i>	6594431	3/03/2022
Standard Sensitivity (SS) RNA kit Part 2 stored at -20°C (Diluent Marker & Intercalating dye)	Enter text.	Enter text.	Enter a date.
<i>Extra Diluent marker</i>	<i>DNF-369-0004</i>	0006602443	7/04/2023
Standard Sensitivity (SS) RNA kit Part 3 stored at -70°C (RNA Ladder)	DNF-382-U020	0006600148	29/03/2022
Capillary conditioning solution	DNF-475-0100	6598614	22/03/2022
DEPC water	AM9961	2004017	N/A
20% Triton-X100 / 30% Ethanol solution	In house	MC1SEP21-01	1/02/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Intercalating dye	Dnf-600-u030	6575146	25/11/2021
Enter text.	Enter text.	Enter text.	Enter a date.

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT ( <b>Drawer B</b> )	18/11/2021	19/11/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT ( <b>Drawer M</b> )	18/11/2021	19/11/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT ( <b>Drawer 3</b> )	18/11/2021	2/12/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	18/11/2021	2/12/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	18/11/2021	20/11/2021  48 hours
<b>Empty waste tray and waste bottle</b> <b>Reagents can be scaled up if required – this table provides the minimum for a single run.</b> <b>Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</b> <b>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</b>  <b>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</b>			

#### 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BF-25	BF-25	BF-25	BF-25	BF-25	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	BF-25	BF-25	BF-25	BF-25	BF-25	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	BF-25	BF-25	BF-25	BF-25	BF-25	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	L

**S1-6** = Samples in triplicate (a, b or c),

**RM** = Reference material

**BF-25** = Blank solution provided in kit,

**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test. Any samples not included in the test must be crossed off the plate layout, and results table below

System Suitability Criteria – RNA Ladder			
Plate location (wells)	D12		
Parameter	Limits	Results	Comments
RNA ladder profile	Visually comparable to figure 4 of SOP	ok	PASS
All peaks present	15 200 500 1000 1500 2000 3000 4000 6000 nt	ok	PASS
Peak heights	<60000 RFU	ok	PASS
Assay Acceptance Criteria – Reference Material			
Plate location (wells)	A12 B12 C12		
LIMS #	2108002914		
BATCH #	EE8493		
EXPIRY	5/02/2022		
Parameter	Limits	Results	Comments
Profile	Visually comparable to DP electropherogram in SOP	Ok/ok/ok	PASS
Migration time	Approximately comparable to profile in SOP	4078/4052/4104	PASS
Lower marker present	LM peak	Ok/ok/ok	PASS
Peak heights	5000-600000 for 2/3 replicates	15553/14183/13671	PASS
No negative peaks or baseline shifts	No significant peaks/shifts	Ok/ok/ok	PASS
Reference Material Dilutions / Calculation / Notes			
thaw date: 08/11/21 270ng/uL = 20 uL of 530 ng/uL master stock + 19 uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution  The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919			

Sample 1 Details	
Plate location (wells)	A11 B11 C11
LIMS #	2111004115
BATCH #	FL5333
EXPIRY	28/02/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4078/4052/ 4078	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	16700/18546/ 17252	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>opened for the first time 15/11/21, stored at 2-8C            270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water            90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919</p>

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 2 Details	
Plate location (wells)	A10 B10 C10
LIMS #	2111004218
BATCH #	FK0738
EXPIRY	30/06/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4027/ 4078/ 4052	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	20552/ 20216/ 18272	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>opened for the first time 18/11/21, stored at 2-8C            270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water            90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919    opened for the first time</p>

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 3 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	PASS
Lower marker	LM must be present	Enter text.	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
Thaw date – 25/10/2021 – stored cell culture fridge , opened for the first time 25/10/21, stored at 2-8C 60 ng/uL = 20 uL of 200 ng/uL DP + 40 uL %Tx100 solution/30% ethanol  The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919

Sample Results	
Choose an item.	
Analysist	s22
Checked by	Enter text.
Sample 4 Details	
Plate location (wells)	A8 B8 C8
LIMS #	2110003932



<b>BATCH #</b>	
<b>EXPIRY</b>	5/05/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	3981/3981/3942	Choose an item.
Lower marker	LM must be present	Ok/ok/ok	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	13683/11870/13713	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	S47	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
<b>Choose an item.</b>	
<b>Analysist</b>	Enter text.
<b>Checked by</b>	Enter text.

Sample 5 Details	
<b>Plate location (wells)</b>	Choose an item.
<b>LIMS #</b>	<b>Click or tap here to enter text.</b>
<b>BATCH #</b>	Click or tap here to enter text.
<b>EXPIRY</b>	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.

Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.
Sample 6 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

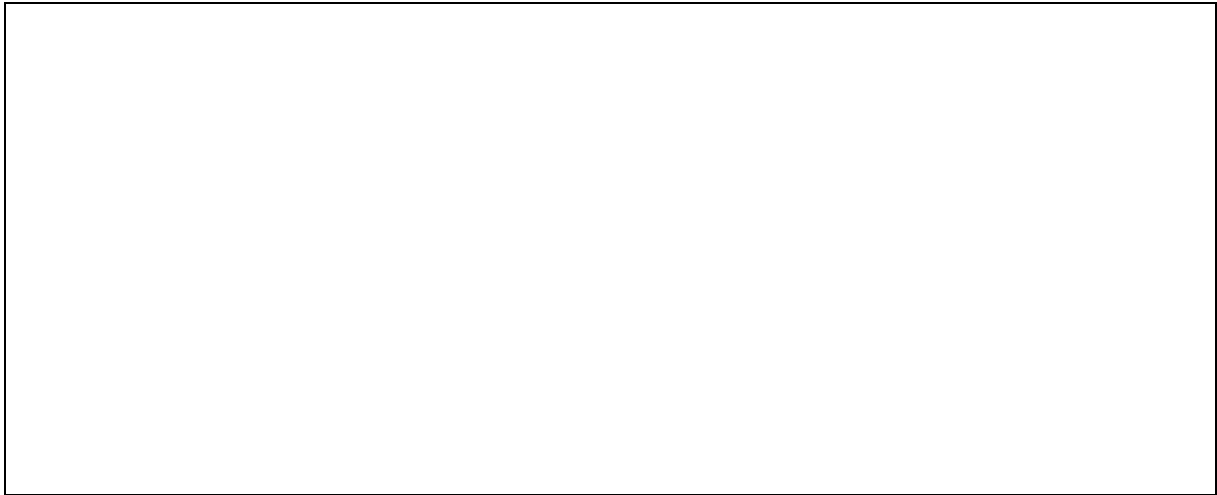
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.

<b>% Late Migrating Species</b>		Enter text.	Enter text.	Enter text.	
---------------------------------	--	-------------	-------------	-------------	--

<b>Sample Dilutions / Calculation / Notes</b>
Enter text.

<b>Sample Results</b>	
<b>Choose an item.</b>	
<b>Analysist</b>	
<b>Checked by</b>	Enter text.

<b>Notes</b>
Enter text.





Owner: s22	Number: Bio-BEE-Form-42
Author: s22	Version: 1
Active: 2/07/2021	Review: <QPulse_DocReviewDate>
Title: Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 18Nov2021

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899LRW Expiry: 18 March 2022Other Reagent: Pyrospense Batch# 0000904583  
November 2021Expiry: 29 June 2022 Use By: 19

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Pfizer Covid vaccine</u>	<u>FK0738</u>	<u>30 June 2022</u>	<u>2111004218-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
<u>n/a</u>	s47	<u>2500</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of vaccine</u>	<u>975uL</u>	<u>5uL Pyrospense</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrospense</u>
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>0.89</u>	s47	<u>0.80</u>	<u>114</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

**Notes:**

**Checked** s22 18Nov2021

Data from Smear Analysis Table

1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate  
 2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the two smear sets.  
 3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	A1	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A1	A1	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
A2	A2	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A2	A2	Blank	4800 nt to 13000 nt	0.0268	58.5	0.0075	11122	11.43
A3	A3	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A3	A3	Blank	4800 nt to 13000 nt	0.213	100	0.0637	10436	11.51
A4	A4	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A4	A4	Blank	4800 nt to 13000 nt	0.0624	100	0.0178	10938	0.87
A5	A5	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A5	A5	Blank	4800 nt to 13000 nt	0.0367	3	0.0118	9670	5.39
A6	A6	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A6	A6	Blank	4800 nt to 13000 nt	0.0837	8.1	0.0238	10971	9.51
A7	A7	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A7	A7	Blank	4800 nt to 13000 nt	0.0969	7.5	0.0262	11544	0.8
A8	A8	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A8	A8	Blank	4800 nt to 13000 nt	0.0308	13	0.0085	11367	0.84
A9	A9	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A9	A9	Blank	4800 nt to 13000 nt	0.0584	5.2	0.0165	11049	1.49
A10	A10	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A10	A10	Blank	4800 nt to 13000 nt	0.0639	0.7	0.0179	11151	1.73
A11	A11	FK6268-2110004						
A11	A11	FK6268-2110004						
B1	B1	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B1	B1	Blank	4800 nt to 13000 nt	0.0887	0.5	0.0251	11029	7.28
B2	B2	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B2	B2	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
B3	B3	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B3	B3	Blank	4800 nt to 13000 nt	0.1945	9	0.0543	11186	0.72
B4	B4	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B4	B4	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
B5	B5	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B5	B5	Blank	4800 nt to 13000 nt	0.0556	4.3	0.0156	11104	0.27
B6	B6	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B6	B6	Blank	4800 nt to 13000 nt	0.0252	25.2	0.0076	10287	20.95
B7	B7	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B7	B7	Blank	4800 nt to 13000 nt	0.0136	7	0.0038	11211	0.18
B8	B8	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B8	B8	Blank	4800 nt to 13000 nt	0.0198	1.5	0.0057	10875	8.53
B9	B9	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B9	B9	Blank	4800 nt to 13000 nt	0.1457	2.1	0.0406	11190	0.34
B10	B10	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
B10	B10	Blank	4800 nt to 13000 nt	0.146	1.8	0.0451	10088	10.84
B11	B11	FK6268-2110004						
B11	B11	FK6268-2110004						
C1	C1	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C1	C1	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
C2	C2	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C2	C2	Blank	4800 nt to 13000 nt	0.0326	0.3	0.0096	10538	12.57
C3	C3	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C3	C3	Blank	4800 nt to 13000 nt	0.002	0.2	0.0006	11153	0.11
C4	C4	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C4	C4	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
C5	C5	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C5	C5	Blank	4800 nt to 13000 nt	0.1054	33.1	0.029	11328	0.38
C6	C6	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C6	C6	Blank	4800 nt to 13000 nt	0.0181	57.4	0.0048	11680	0.83
C7	C7	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C7	C7	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
C8	C8	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C8	C8	Blank	4800 nt to 13000 nt	0.0122	12.8	0.0033	11455	1.36
C9	C9	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C9	C9	Blank	4800 nt to 13000 nt	0.3963	20	0.1081	11437	0.48
C10	C10	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
C10	C10	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
C11	C11	FK6268-2110004						
C11	C11	FK6268-2110004						
D1	D1	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D1	D1	Blank	4800 nt to 13000 nt	0.0084	0.8	0.0026	10036	2.81
D2	D2	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D2	D2	Blank	4800 nt to 13000 nt	0.3879	6.6	0.1074	11263	2.39
D3	D3	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D3	D3	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
D4	D4	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D4	D4	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
D5	D5	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D5	D5	Blank	4800 nt to 13000 nt	0	0	0	10503	0
D6	D6	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D6	D6	Blank	4800 nt to 13000 nt	0.0166	5.8	0.0048	10897	1.13
D7	D7	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D7	D7	Blank	4800 nt to 13000 nt	0.0113	100	0.0032	10890	1.67
D8	D8	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D8	D8	Blank	4800 nt to 13000 nt	0.0366	100	0.0105	10845	4.32
D9	D9	Blank	3700 nt to 4800 nt	0.0066	0.2	0.0052	3981	3.08
D9	D9	Blank	4800 nt to 13000 nt	0.0603	1.6	0.0236	7952	22.46
D10	D10	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D10	D10	Blank	4800 nt to 13000 nt	0.151	100	0.0478	9861	7.06
D11	D11	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
D11	D11	Blank	4800 nt to 13000 nt	0.0342	100	0.01	10657	0.63
D12	D12	Ladder						





3	C9	Blank	4800 nt to 13000 nt	0.3963	20	0.1081	11437	0.48				
1	A10	Blank	4800 nt to 13000 nt	0.0639	0.7	0.0179	11151	1.73				
2	B10	Blank	4800 nt to 13000 nt	0.146	1.8	0.0451	10088	10.84	Blank	0.83	0.91	108.89
3	C10	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN				
1	A11	FK6268-2110004031	4800 nt to 13000 nt	0.0639	0.7	0.0179	11151	1.73				
2	B11	FK6268-2110004031	4800 nt to 13000 nt	0.146	1.8	0.0451	10088	10.84	FK6268-2110004031	0.83	0.91	108.89
3	C11	FK6268-2110004031	4800 nt to 13000 nt	0	0	NaN	NaN	NaN				
1	D1	Blank	4800 nt to 13000 nt	0.0084	0.8	0.0026	10036	2.81				
2	D2	Blank	4800 nt to 13000 nt	0.3879	6.6	0.1074	11263	2.39	Blank	3.70	4.10	110.84
3	D3	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN				
1	D4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN				
2	D5	Blank	4800 nt to 13000 nt	0	0	0	10503	0	Blank	1.93	3.35	173.21
3	D6	Blank	4800 nt to 13000 nt	0.0166	5.8	0.0048	10897	1.13				
1	D7	Blank	4800 nt to 13000 nt	0.0113	100	0.0032	10890	1.67				
2	D8	Blank	4800 nt to 13000 nt	0.0366	100	0.0105	10845	4.32	Blank	67.20	56.81	84.54
3	D9	Blank	4800 nt to 13000 nt	0.0603	1.6	0.0236	7952	22.46				

This tab is only to be used if a replace needs to be excluded from the analysis.

Enter raw data directly into this table. All averages/ pass fail will be calculated automatically using the pass/fail parameters entered in the Calculations tab.

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS	
									Sample ID	Average	stdev	%CV		
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

Pass/Fail Parameters		
minimum	cut off	maximum
\$47		
result >>	\$47	

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
									Sample ID	Average	stdev	%CV	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	





## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2021 11 09\12-26-20\2021 11 09 12H 26M.raw

**Created:** Tuesday, November 9, 2021 12:51:46 PM

**Number of capillaries:** 10

**Array serial number:** 022621-27SFS

**Effect length:** 33 cm

**Array usage count:** 34

**Instrument type:** 5300 Fragment Analyzer

**Instrument controller software version:** 3.1.0.12

**Device serial number:** MY2105AB19

## Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds

**Gel prime:** No

**Full conditioning:** Yes

**Gel prime to buffer:** Yes

**Gel selection:** Gel 2

**Perform prerun:** 8.0 kV, 30 sec.

**Rinse:** No

**Marker 1:** No

**Rinse:** Tray: 3, Row: A, Dip count: 2

**Sample injection:** 5.0 kV, 6 sec.

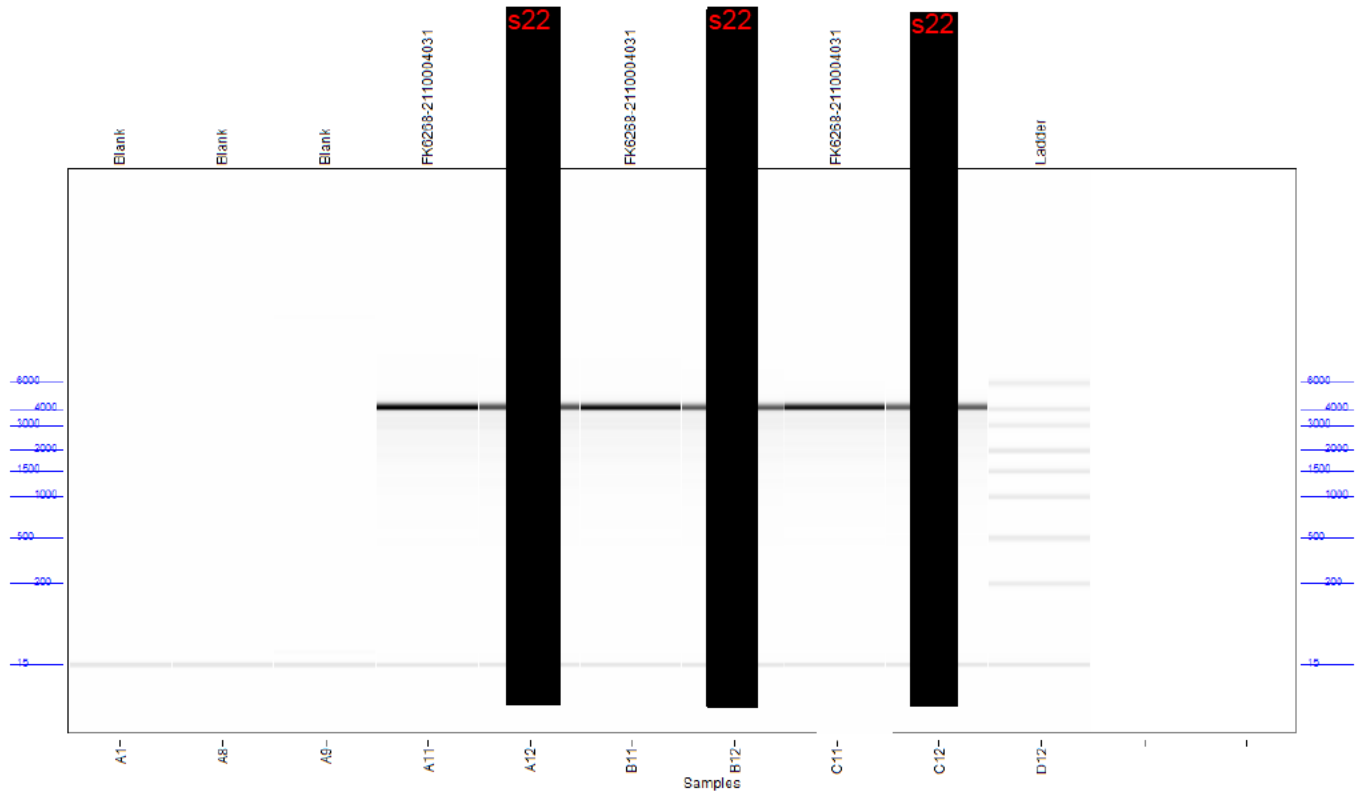
**Separation:** 8.0 kV, 60.0 min.

**Tray name:** Tray-1

**Analysis mode:** RNA (Eukaryotic)

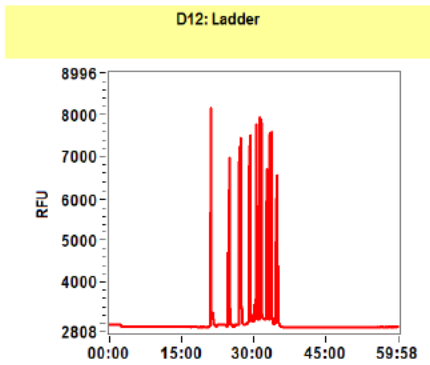
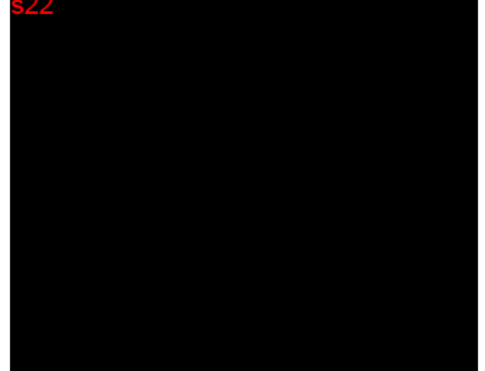
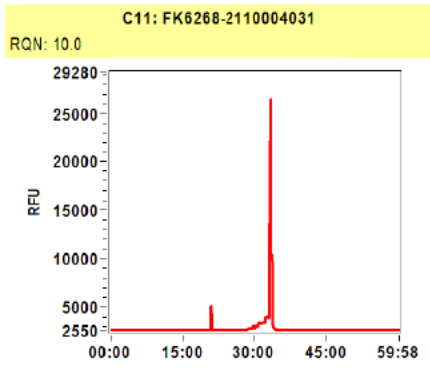
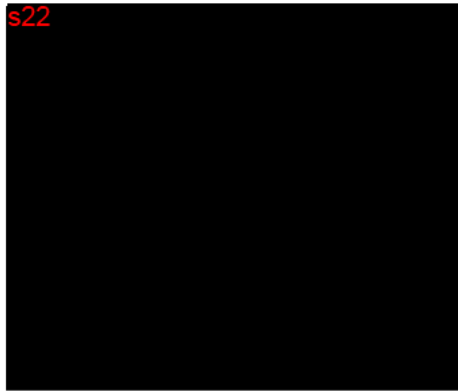
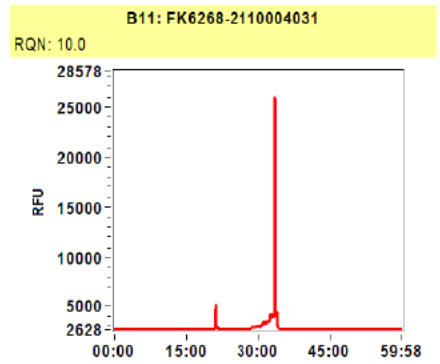
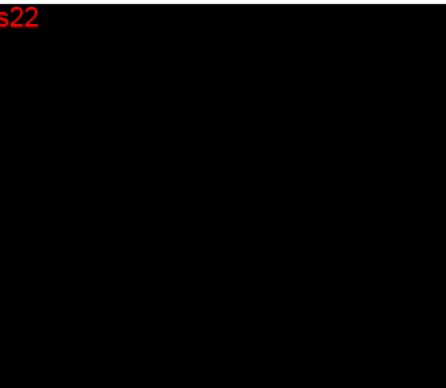
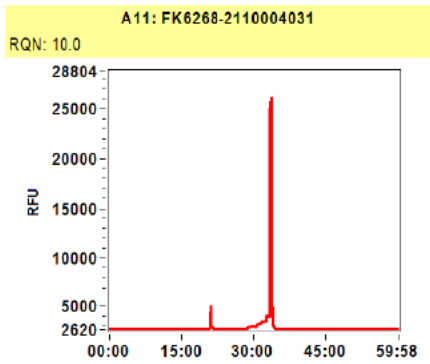
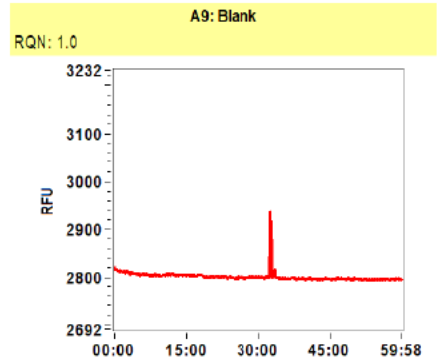
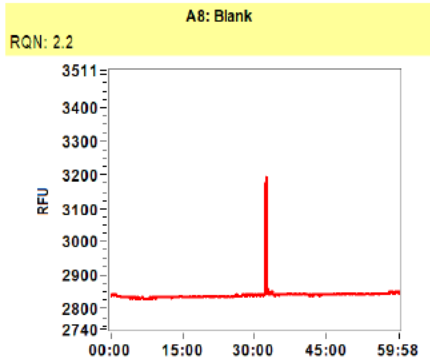
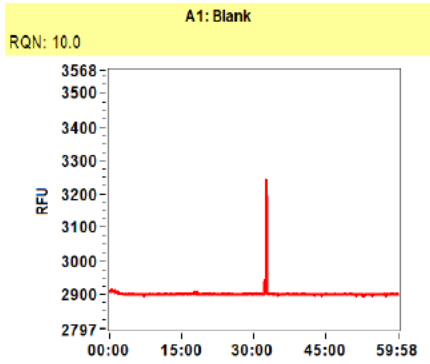
## Notes

### Gel Image

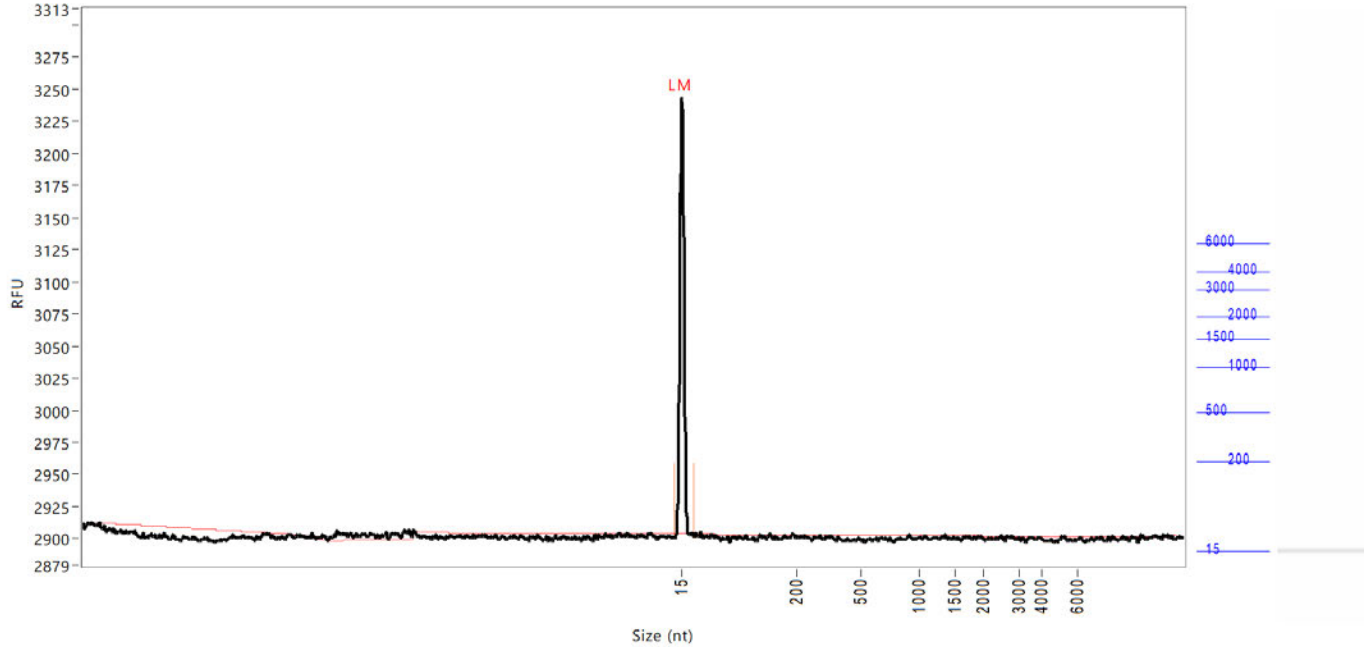


s22

s22



**Sample:** Blank  
**Well location:** A1  
**Created:** Tuesday, November 9, 2021 12:51:46 PM



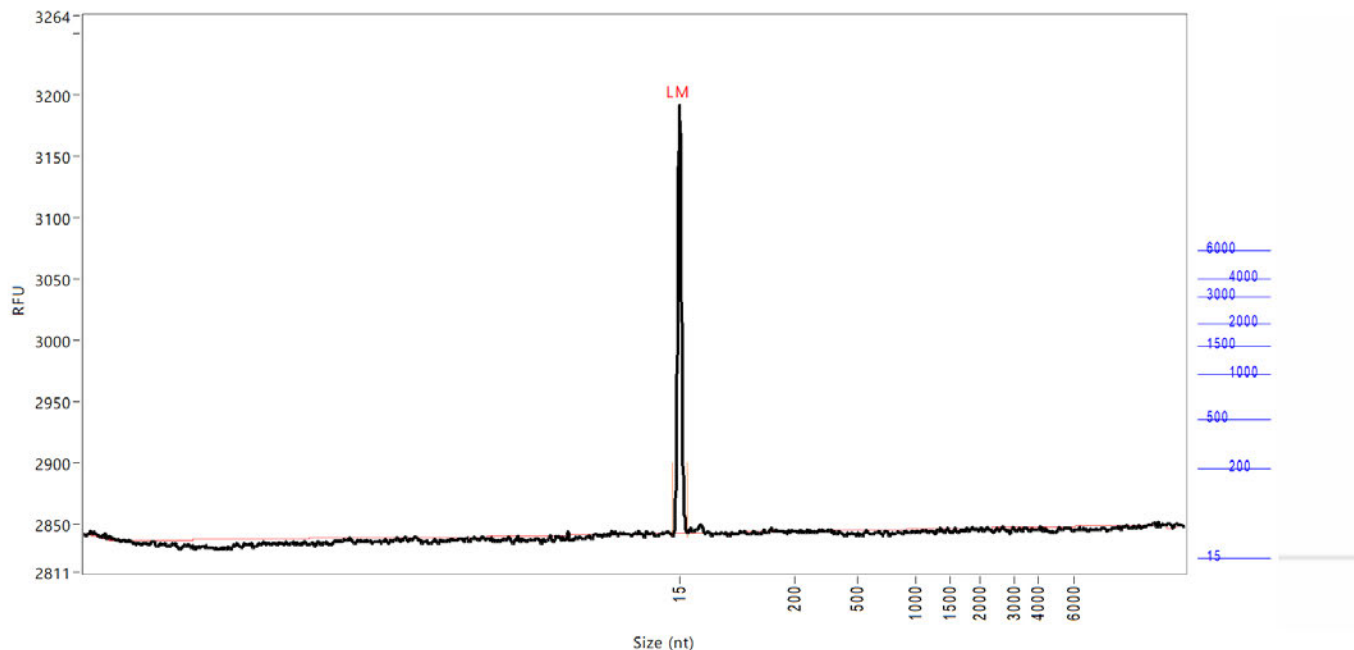
Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	1.1588	4	35	338
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0000	ng/uL		
	28s/18s:	0.0			
	RQN	10.0			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	NaN %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0000 ng/ul	NaN %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2



**Sample:** Blank  
**Well location:** A8  
**Created:** Tuesday, November 9, 2021 12:51:46 PM

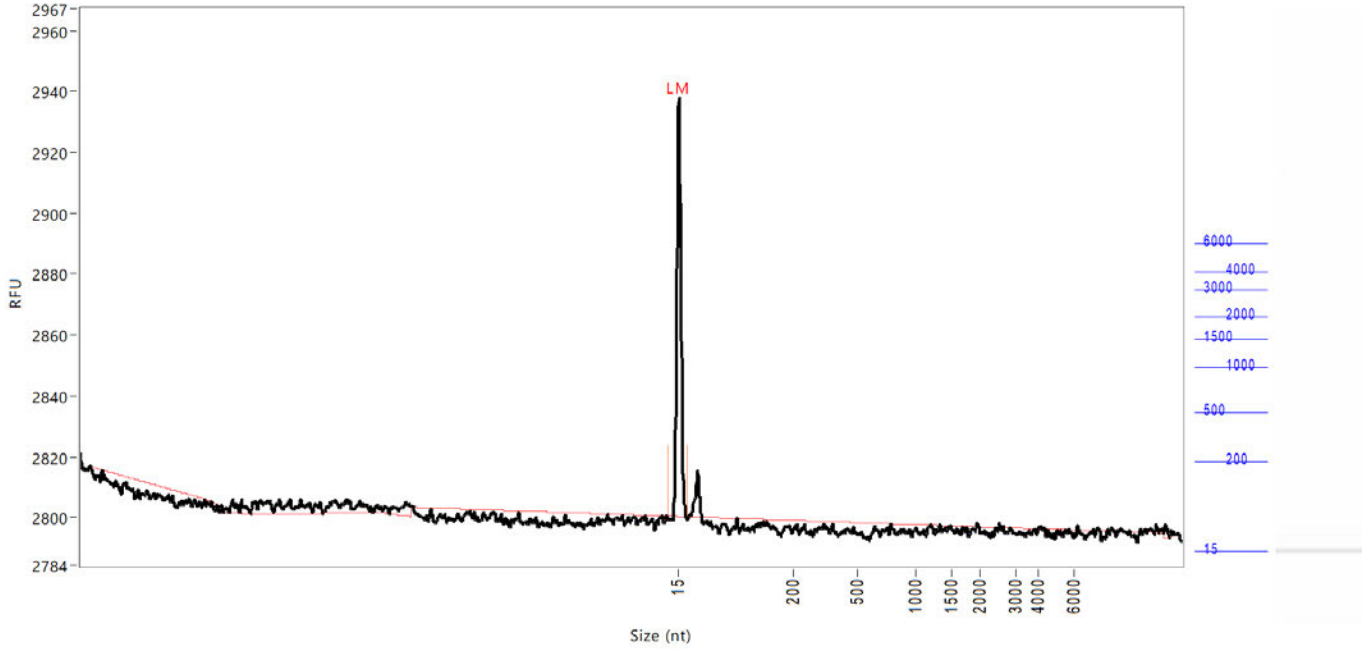


Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	1.1588	4	28	347
TIC:		0.0000	ng/uL		
TIM:		0.0000	nmole/L		
Total concentration:		0.2362	ng/uL		
28s/18s:		0.0			
RQN		2.2			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0308 ng/ul	13.0 %Total	0.0085 nmole/L	11367 Avg. Size (nt)	0.84 %CV

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Blank  
**Well location:** A9  
**Created:** Tuesday, November 9, 2021 12:51:46 PM

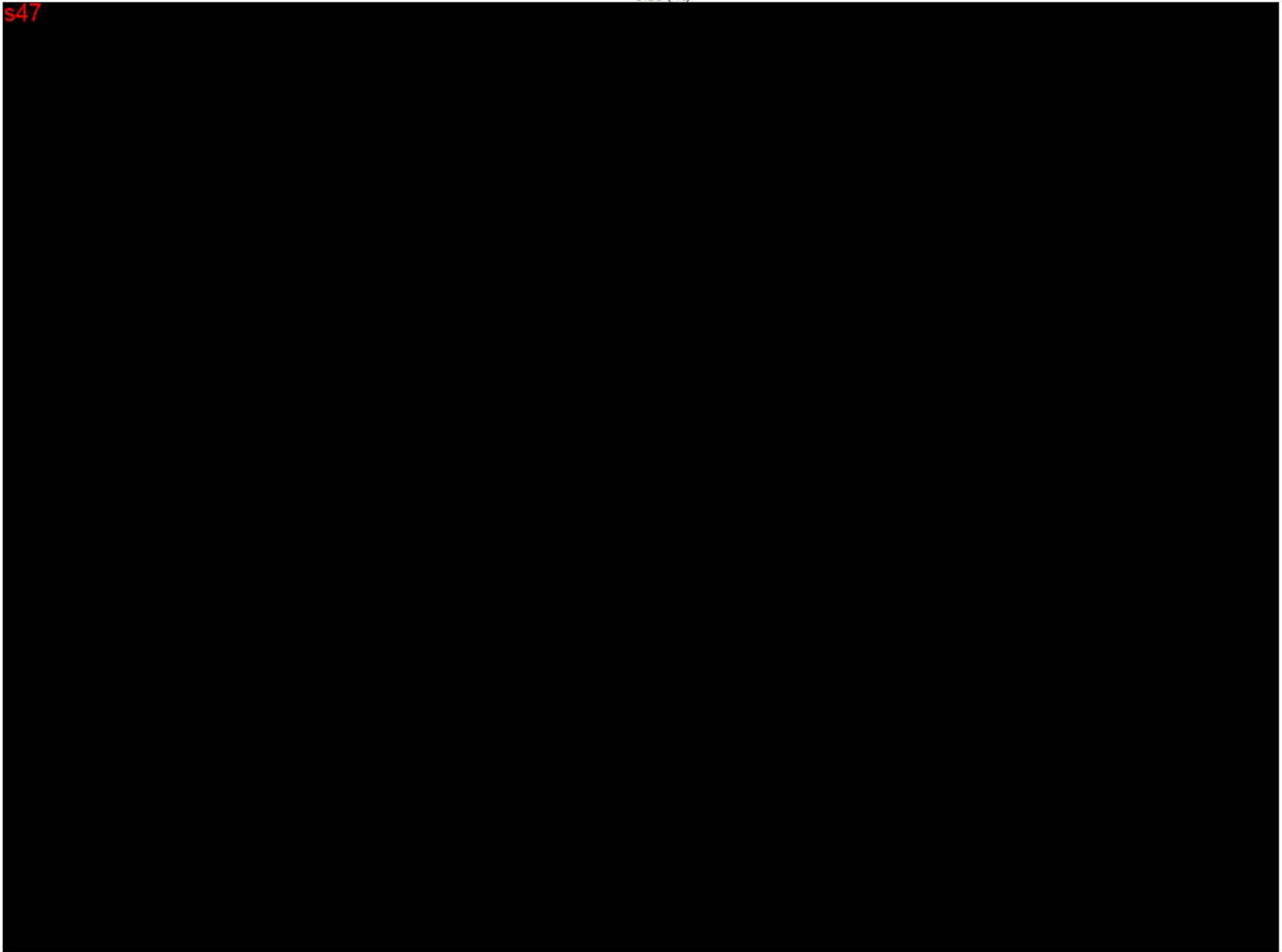
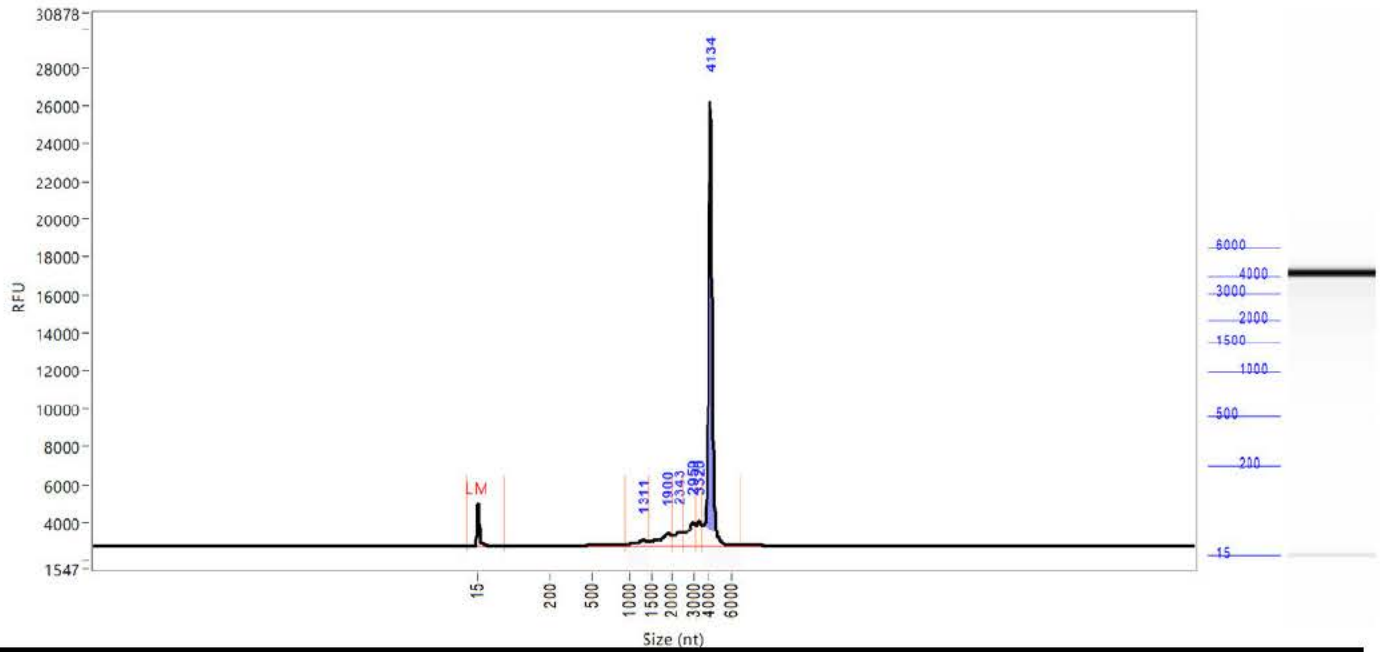
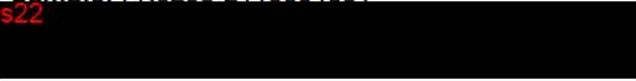


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	1.1588	0	28	136
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	1.1340	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0584 ng/ul	5.2 %Total	0.0165 nmole/L	11049 Avg. Size (nt)	1.49 %CV

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

Sample: EK6268-2110004031

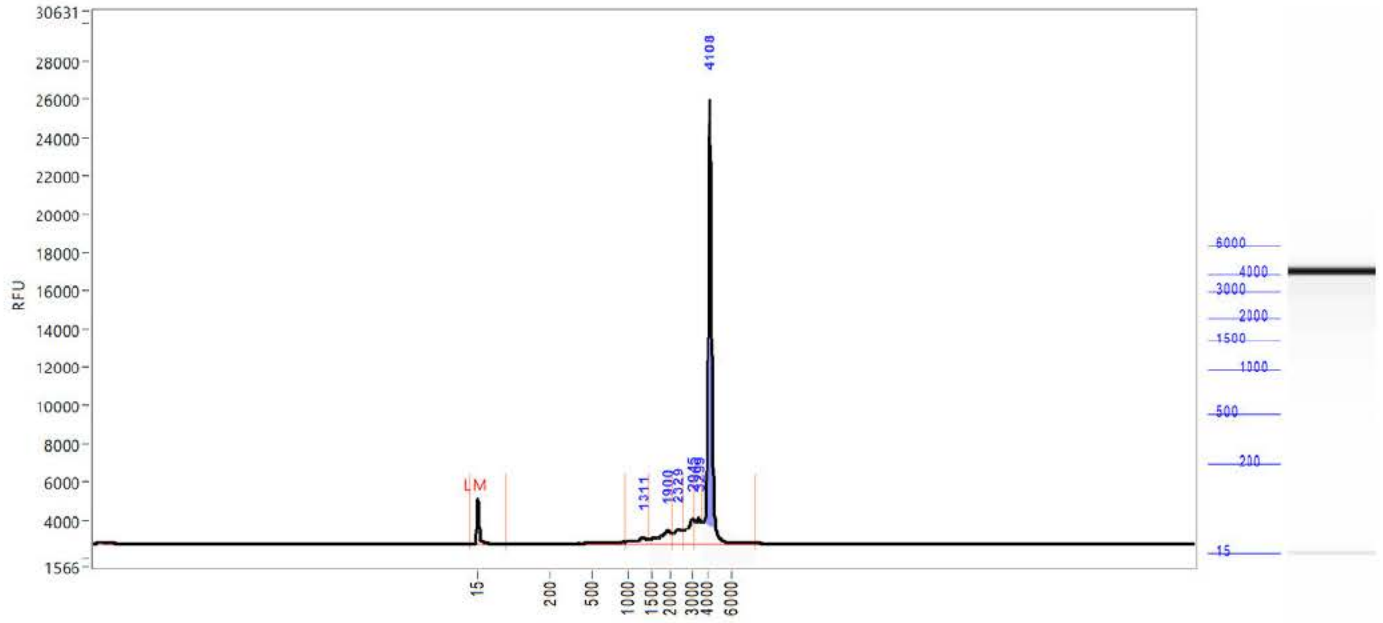


s22

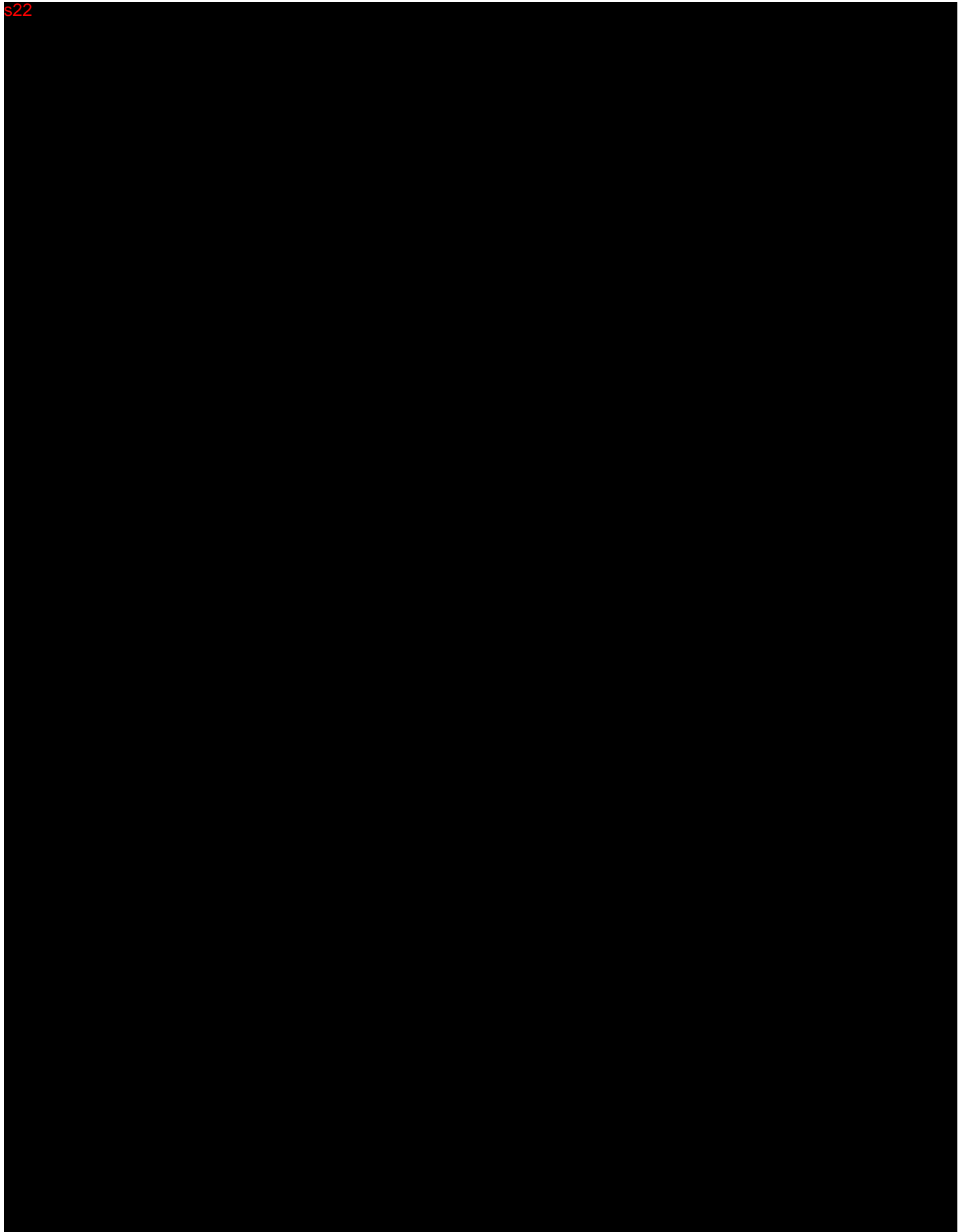


Sample: FK6268-2110004031

s22



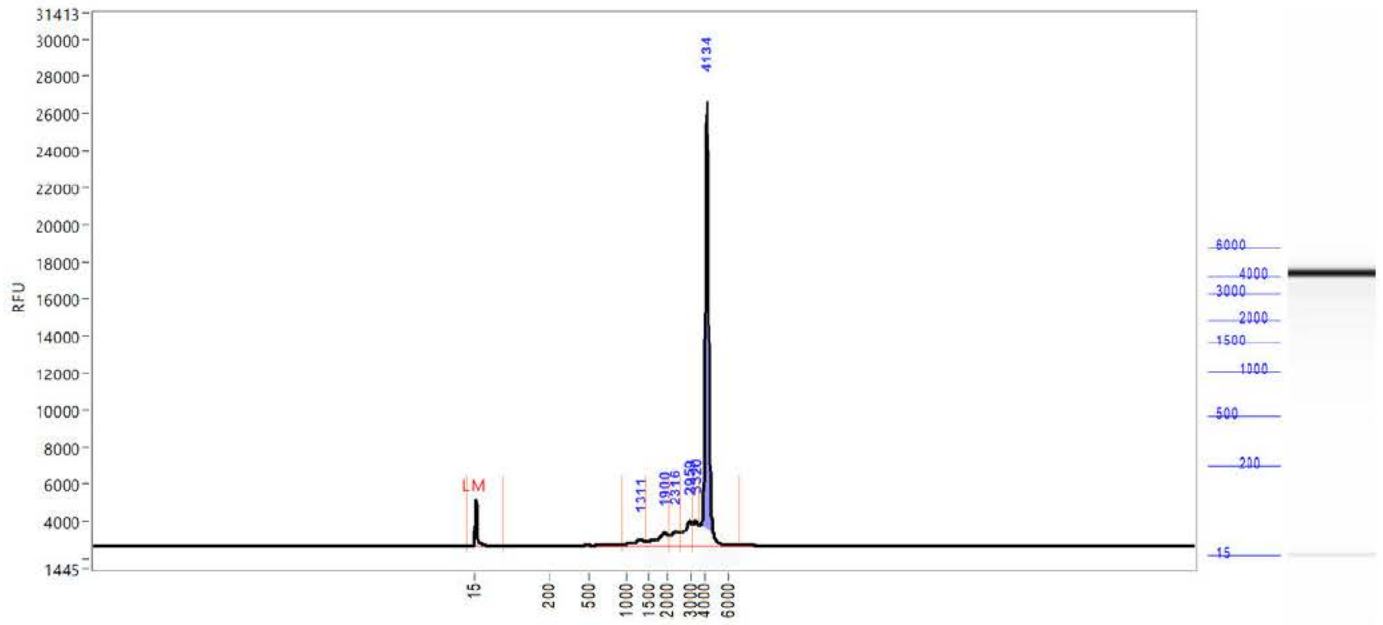
s47



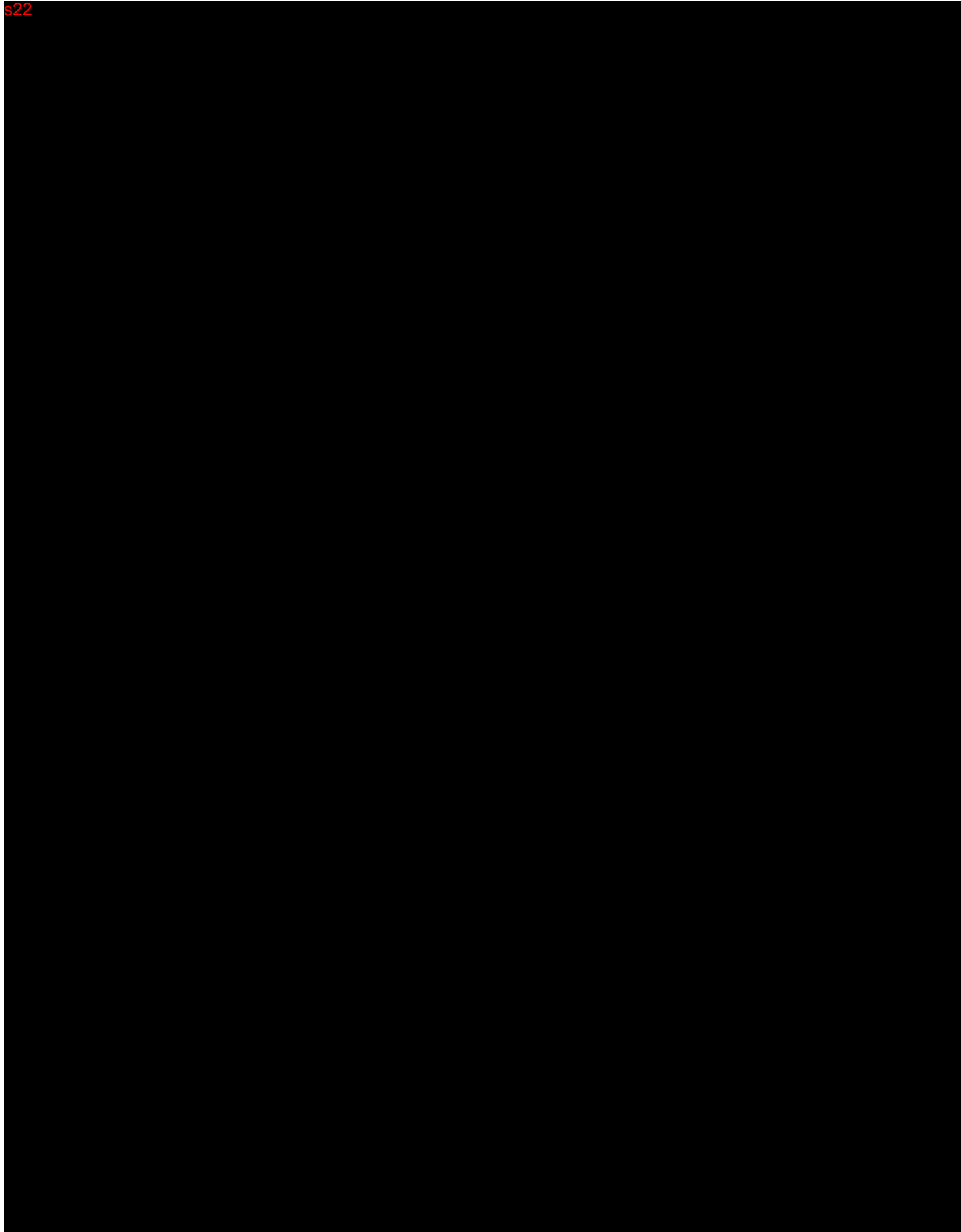
s22

Sample: FK6268-2110004031

s22



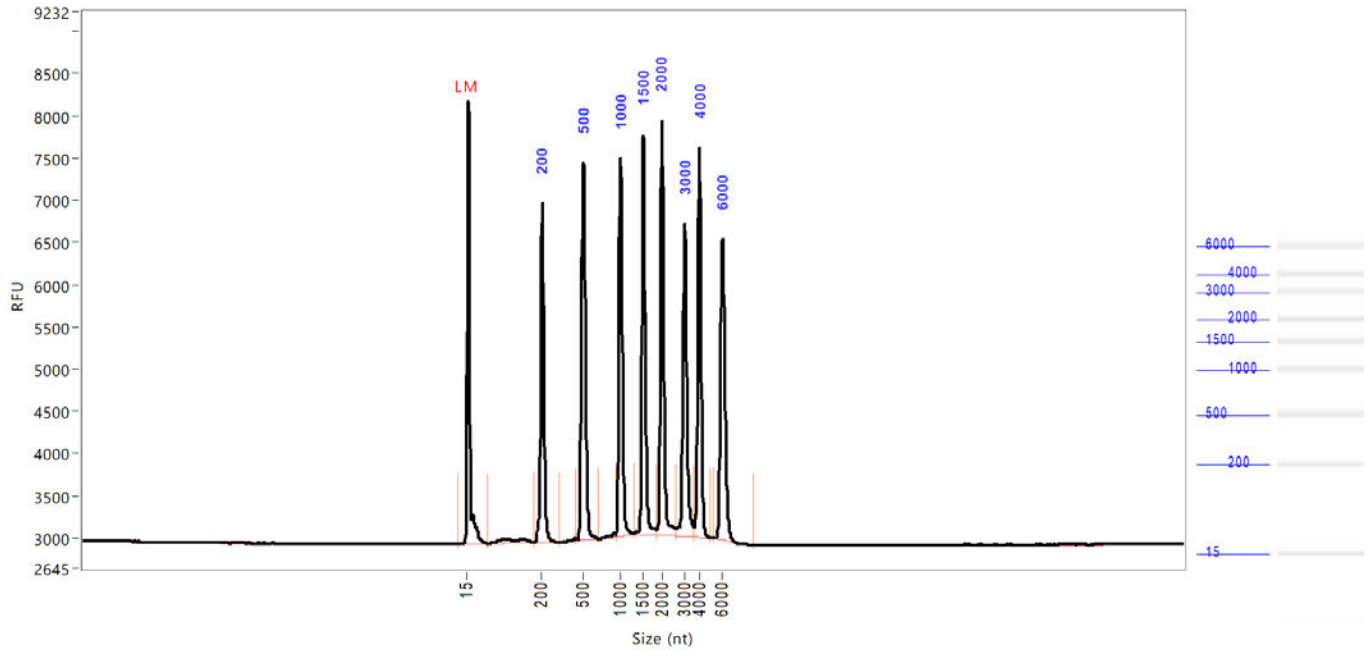
s47



s22



**Sample:** Ladder  
**Well location:** D12  
**Created:** Tuesday, November 9, 2021 12:51:46 PM



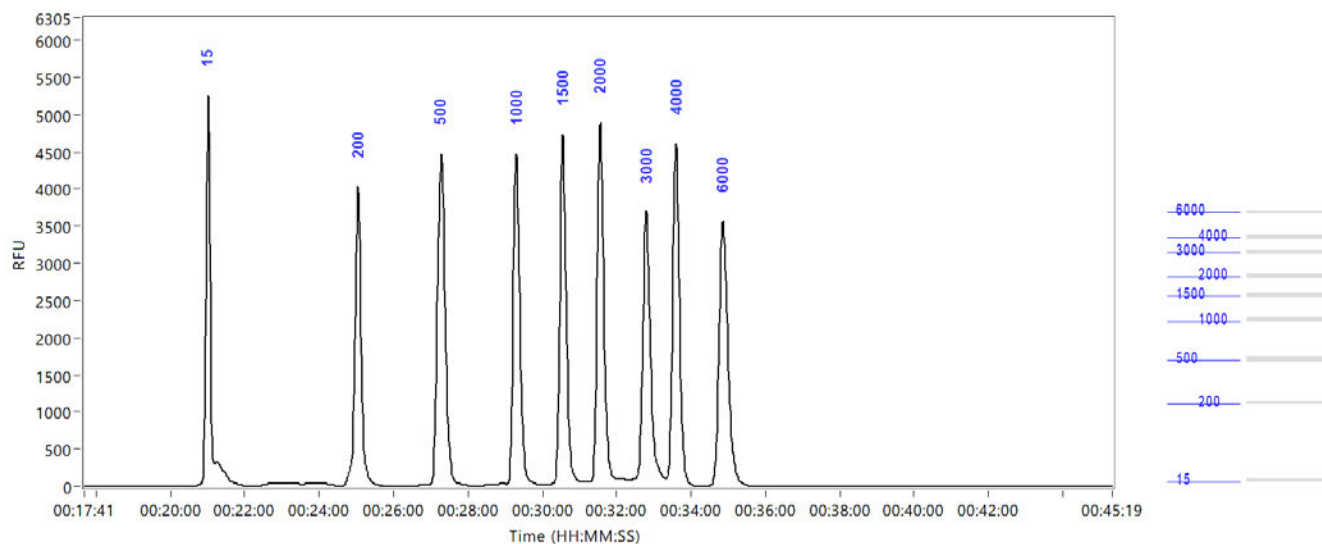
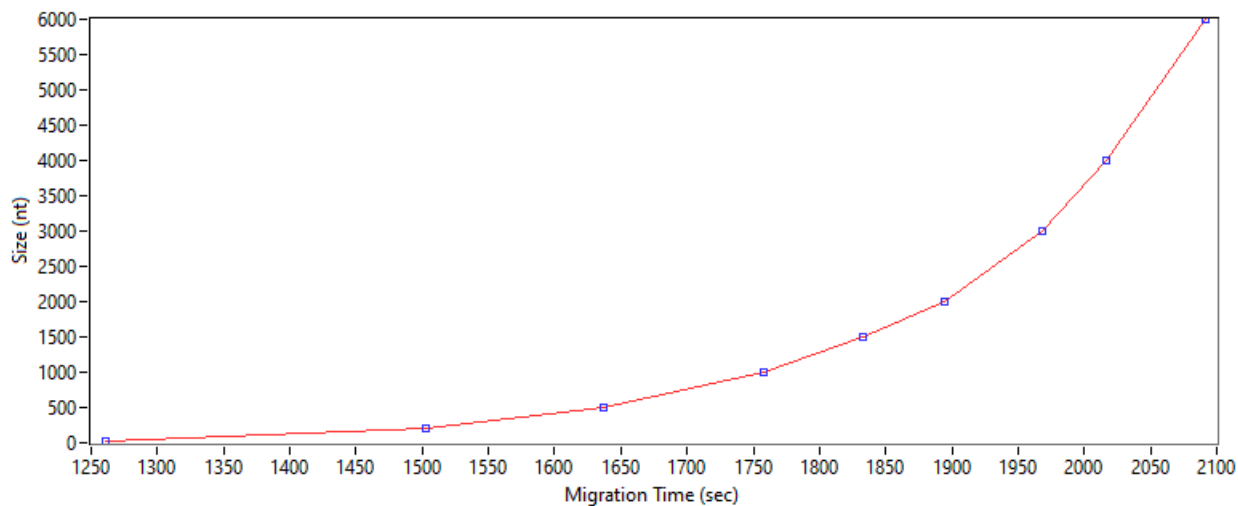
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	1.1588	0	66	5252
2	200	11.7968	180	325	4025
3	500	15.1062	453	713	4463
4	1000	11.9995	938	1291	4475
5	1500	11.9449	1291	1859	4723
6	2000	12.6914	1859	2617	4896
7	3000	10.4944	2617	3639	3698
8	4000	11.0108	3639	4934	4603
9	6000	10.7683	5254	8633	3568

TIC: 95.8123 ng/uL  
 TIM: 381.6487 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6      Sample min peak height: 200      Sample baseline V to V?: Y      Sample baseline V to V points: 3  
 Sample filter: Binomial      Number of points for filter: 9      Sample start region (min): 0      Sample end region (min): 60  
 Marker peak width (sec): 6      Marker min peak height: 100      Marker baseline V to V?: Y      Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU      Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder      Final concentration (ng/uL): 8.0000      Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Tuesday, November 9, 2021 12:51:46 PM  
**Fit type:** Point to point

Calibration curve





Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Appendix 1 - Fragment Analyzer Worksheet - Pfizer COMIRNATY	

### Worksheet for Fragment Analyzer - RNA Integrity

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-26	<b>Analysist</b>	s22
<b>TRIM link to data files</b>	D21-3309192,D21-3309189	<b>Test Date</b>	9/11/2021

Pipettes & Equipment	
Name	LIMS#
30-300 µL 12 channel pipette	N/A
p10 pipette	32835
p50 pipette	N/A
p100 pipette	32792
p200 pipette	5649
Thermomixer	23660
Thermocycler	N/A
P20	32891

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	N/A
Standard Sensitivity (SS) RNA kit Part 1 stored at 2-8°C	DNF-471-0500	0006609959	10/03/2022
<i>Extra Blank solution</i>	DNF-300-0008	6594431	3/03/2022
Standard Sensitivity (SS) RNA kit Part 2 stored at -20°C (Diluent Marker & Intercalating dye)	Enter text.	Enter text.	Enter a date.
<i>Extra Diluent marker</i>	DNF-369-0004	0006602443	7/04/2023
Standard Sensitivity (SS) RNA kit Part 3 stored at -70°C (RNA Ladder)	DNF-382-U020	0006600148	29/03/2022
Capillary conditioning solution	DNF-475-0100	6598614	22/03/2022
DEPC water	AM9961	2004017	N/A
20% Triton-X100 / 30% Ethanol solution	In house	MC1SEP21-01	1/02/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Intercalating dye	Dnf-600-u030	6603014	9/04/2022
Enter text.	Enter text.	Enter text.	Enter a date.

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT (Drawer B)	9/11/2021	10/11/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT (Drawer M)	9/11/2021	10/11/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT (Drawer 3)	8/11/2021	22/11/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	9/11/2021	23/11/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	9/11/2021	11/11/2021  48 hours
<b>Empty waste tray and waste bottle</b> <b>Reagents can be scaled up if required – this table provides the minimum for a single run.</b> <b>Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</b> <b>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</b>  <b>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</b>			

## 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BF-25	BF-25	BF-25	BF-25	BF-25	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	BF-25	BF-25	BF-25	BF-25	BF-25	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	BF-25	BF-25	BF-25	BF-25	BF-25	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	L

S1-6 = Samples in triplicate (a, b or c),

RM = Reference material

BF-25 = Blank solution provided in kit,

L = RNA ladder

This worksheet assumes the maximum of 6 samples per test. Any samples not included in the test must be crossed off the plate layout, and results table below

System Suitability Criteria – RNA Ladder			
Plate location (wells)	D12		
Parameter	Limits	Results	Comments
RNA ladder profile	Visually comparable to figure 4 of SOP	ok	PASS
All peaks present	15 200 500 1000 1500 2000 3000 4000 6000 nt	ok	PASS
Peak heights	<60000 RFU	ok	PASS
Assay Acceptance Criteria – Reference Material			
Plate location (wells)	A12 B12 C12		
LIMS #	2108002914		
BATCH #	EE8493		
EXPIRY	5/02/2022		
Parameter	Limits	Results	Comments
Profile	Visually comparable to DP electropherogram in SOP	Ok/ok/ok	PASS
Migration time	Approximately comparable to profile in SOP	4134/4108/4134	PASS
Lower marker present	LM peak	Ok/ok/ok	PASS
Peak heights	5000-600000 for 2/3 replicates	18286/16461/15492	PASS
No negative peaks or baseline shifts	No significant peaks/shifts	Ok/ok/ok	PASS
Reference Material Dilutions / Calculation / Notes			
thaw date: 08/11/21 270ng/uL = 20 uL of 530 ng/uL master stock + 19 uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution  The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919			

Sample 1 Details	
Plate location (wells)	A11 B11 C11
LIMS #	2110004031
BATCH #	FK6268
EXPIRY	28/02/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4134/4108/4134	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	23447/23229/23956	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>opened for the first time 08/11/21, stored at 2-8C                      270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water                      90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919</p>

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 2 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	S47	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.
Sample 3 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	PASS
Lower marker	LM must be present	Enter text.	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
Thaw date – 25/10/2021 – stored cell culture fridge , opened for the first time 25/10/21, stored at 2-8C 60 ng/uL = 20 uL of 200 ng/uL DP + 40 uL %Tx100 solution/30% ethanol  The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919

Sample Results	
<b>Choose an item.</b>	
Analysist	s22
Checked by	Enter text.
Sample 4 Details	
Plate location (wells)	A8 B8 C8
LIMS #	2110003932
BATCH #	s22
EXPIRY	5/05/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	3981/3981/3942	Choose an item.
Lower marker	LM must be present	Ok/ok/ok	Choose an item.



Peak heights	5000-60000 RFU for 2/3 replicates	13683/11870/13713	Choose an item.
--------------	--------------------------------------	-------------------	-----------------

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

Sample 5 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.

<b>% Late Migrating Species</b>		Enter text.	Enter text.	Enter text.	
---------------------------------	--	-------------	-------------	-------------	--

Sample Dilutions / Calculation / Notes					
Enter text.					

Sample Results					
<b>Choose an item.</b>					
<b>Analysist</b>			Enter text.		
<b>Checked by</b>			Enter text.		
Sample 6 Details					
<b>Plate location (wells)</b>	Choose an item.				
<b>LIMS #</b>	<b>Click or tap here to enter text.</b>				
<b>BATCH #</b>	Click or tap here to enter text.				
<b>EXPIRY</b>	Enter date.				

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
<b>% RNA Integrity</b>	s47	Enter text.	Enter text.	Enter text.	Choose an item.
<b>% Late Migrating Species</b>		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes					
Enter text.					

Sample Results					
<b>Choose an item.</b>					

<b>Analysist</b>	
<b>Checked by</b>	Enter text.

<b>Notes</b>
Enter text.





Owner: s22	Number: Bio-BEE-Form-42
Author: s22	Version: 1
Active: 2/07/2021	Review: <QPulse_DocReviewDate>
Title: Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 08Nov2021/2

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899LRW Expiry: 18 March 2022Other Reagent: Pyrospense Batch# 0000904583  
November 2021Expiry: 29 June 2022 Use By: 19

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Pfizer Comirnaty Covid vaccine</u>	<u>FK6268</u>	<u>28 February 2022</u>	<u>2110004031-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
<u>n/a</u>	s47	<u>2500</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of vaccine</u>	<u>975uL</u>	<u>5uL Pyrospense</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrospense</u>
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>4.46</u>	s47	<u>0.55</u>	<u>128</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

**Notes:**

**Checked** s22 **9Nov2021**

## Data from Smear Analysis Table

1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate  
 2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the two smear sets.  
 3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	A1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A1	A1	Blank	4800 nt to 13000 nt	0.0114	14.1	0.0037	9597	3.84
A2	A2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A2	A2	Blank	4800 nt to 13000 nt	0.0094	53.6	0.003	9733	2.16
A3	A3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A3	A3	Blank	4800 nt to 13000 nt	0.0028	0.1	0.0009	9401	0.33
A4	A4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A4	A4	Blank	4800 nt to 13000 nt	0.0132	1.6	0.0044	9330	3.99
A5	A5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A5	A5	Blank	4800 nt to 13000 nt	0.0284	28.2	0.0104	8534	1.12
A6	A6	Blank	3700 nt to 4800 nt	0.0055	2.8	0.0046	3769	0.49
A6	A6	Blank	4800 nt to 13000 nt	0.0463	23.6	0.0168	8586	9.8
A7	A7	Blank	3700 nt to 4800 nt	0.0692	0.9	0.0568	3802	2.82
A7	A7	Blank	4800 nt to 13000 nt	0.3611	4.9	0.129	8731	12.17
A8	A8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A8	A8	Blank	4800 nt to 13000 nt	0.0114	99.8	0.0038	9416	5.51
A9	A9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A9	A9	Blank	4800 nt to 13000 nt	0.0018	10.4	0.0006	8785	6.73
A10	A10	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A10	A10	Blank	4800 nt to 13000 nt	0.0759	1.6	0.0271	8728	5.33
A11	A11	FL7649-2110003						
A11	A11	FL7649-2110003						
A12	A12							
A12	A12							
B1	B1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B1	B1	Blank	4800 nt to 13000 nt	0.0035	7.8	0.0012	9317	4.66
B2	B2	Blank	3700 nt to 4800 nt	0.0044	0	0.0035	3849	1.31
B2	B2	Blank	4800 nt to 13000 nt	0.3856	3.7	0.1417	8487	11.19
B3	B3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B3	B3	Blank	4800 nt to 13000 nt	0.0116	31.8	0.0041	8795	7.75
B4	B4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B4	B4	Blank	4800 nt to 13000 nt	0.0007	2.6	0.0002	9674	0.12
B5	B5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B5	B5	Blank	4800 nt to 13000 nt	0.0004	38.8	0.0001	8615	0.14
B6	B6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B6	B6	Blank	4800 nt to 13000 nt	0.0051	98.3	0.0018	8971	6.34
B7	B7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B7	B7	Blank	4800 nt to 13000 nt	0.023	28.3	0.0081	8797	5
B8	B8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B8	B8	Blank	4800 nt to 13000 nt	0.0038	97	0.0014	8606	5.35
B9	B9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B9	B9	Blank	4800 nt to 13000 nt	0.0102	100	0.0036	8732	7.41
B10	B10	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B10	B10	Blank	4800 nt to 13000 nt	0.007	5.4	0.0026	8591	6.52
B11	B11	FL7649-2110003						
B11	B11	FL7649-2110003						
B12	B12							
B12	B12							
C1	C1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C1	C1	Blank	4800 nt to 13000 nt	0.0065	79	0.0023	8752	2.85
C2	C2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C2	C2	Blank	4800 nt to 13000 nt	0.0119	100	0.0041	9078	5.02
C3	C3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C3	C3	Blank	4800 nt to 13000 nt	0.0057	83.5	0.002	8781	2.76
C4	C4	Blank	3700 nt to 4800 nt	0.0007	0.1	0.0006	3953	0.24
C4	C4	Blank	4800 nt to 13000 nt	0.0749	11.8	0.0287	8143	16.11
C5	C5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C5	C5	Blank	4800 nt to 13000 nt	0.0072	80.4	0.0024	9187	4.33
C6	C6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C6	C6	Blank	4800 nt to 13000 nt	0.0012	100	0.0004	10334	0.76
C7	C7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C7	C7	Blank	4800 nt to 13000 nt	0.0092	73.3	0.0032	8941	2.82
C8	C8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C8	C8	Blank	4800 nt to 13000 nt	0.0079	51.9	0.0026	9602	5.12
C9	C9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C9	C9	Blank	4800 nt to 13000 nt	0.0046	1.9	0.0015	9863	1.36
C10	C10	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C10	C10	Blank	4800 nt to 13000 nt	0.0006	0.9	0.0002	9106	7.61
C11	C11	FL7649-2110003						
C11	C11	FL7649-2110003						
C12	C12							
C12	C12							
D1	D1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D1	D1	Blank	4800 nt to 13000 nt	0.0097	2.9	0.0034	9026	1.22
D2	D2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	4800 nt to 13000 nt	0.0911	8.7	0.0315	9016	2.57
D3	D3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	4800 nt to 13000 nt	0.0191	34.2	0.0068	8790	4.69
D4	D4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	4800 nt to 13000 nt	0.0035	55.3	0.0012	8702	0.82
D5	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	4800 nt to 13000 nt	0.001	100	0.0004	8320	0.26
D6	D6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D6	D6	Blank	4800 nt to 13000 nt	0.0008	63.4	0.0003	8544	1.67
D7	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	4800 nt to 13000 nt	0.0046	100	0.0016	8683	0.44
D8	D8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	4800 nt to 13000 nt	0.0089	100	0.0031	8824	2.61
D9	D9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D9	D9	Blank	4800 nt to 13000 nt	0	0	0	9186	0.09
D10	D10	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D10	D10	Blank	4800 nt to 13000 nt	0.021	1.7	0.0082	7984	12.41
D11	D11	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D11	D11	Blank	4800 nt to 13000 nt	0.0101	99.1	0.0035	8952	4.68
D12	D12	Ladder						
D12	D12							





3	C9	Blank	4800 nt to 13000 nt	0.0046	1.9	0.0015	9863	1.36				
1	A10	Blank	4800 nt to 13000 nt	0.0759	1.6	0.0271	8728	5.33				
2	B10	Blank	4800 nt to 13000 nt	0.007	5.4	0.0026	8591	6.52	Blank	2.63	2.42	91.95
3	C10	Blank	4800 nt to 13000 nt	0.0006	0.9	0.0002	9106	7.61				
1	A11	FL7649-2110003905										
2	B11	FL7649-2110003905							FL7649-2110003905			
3	C11	FL7649-2110003905										
1	D1	Blank	4800 nt to 13000 nt	0.0097	2.9	0.0034	9026	1.22				
2	D2	Blank	4800 nt to 13000 nt	0.0911	8.7	0.0315	9016	2.57	Blank	15.27	16.65	109.07
3	D3	Blank	4800 nt to 13000 nt	0.0191	34.2	0.0068	8790	4.69				
1	D4	Blank	4800 nt to 13000 nt	0.0035	55.3	0.0012	8702	0.82				
2	D5	Blank	4800 nt to 13000 nt	0.001	100	0.0004	8320	0.26	Blank	72.90	23.82	32.67
3	D6	Blank	4800 nt to 13000 nt	0.0008	63.4	0.0003	8544	1.67				
1	D7	Blank	4800 nt to 13000 nt	0.0046	100	0.0016	8683	0.44				
2	D8	Blank	4800 nt to 13000 nt	0.0089	100	0.0031	8824	2.61	Blank	66.67	57.74	86.60
3	D9	Blank	4800 nt to 13000 nt	0	0	0	9186	0.09				

This tab is only to be used if a replace needs to be excluded from the analysis.

Enter raw data directly into this table. All averages/ pass fail will be calculated automatically using the pass/fail parameters entered in the Calculations tab.

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS	
									Sample ID	Average	stdev	%CV		
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

Pass/Fail Parameters	
\$47	
result >>	\$47

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
									Sample ID	Average	stdev	%CV	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	

### VALIDATION DATA

Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2
A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4
A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4
A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6
A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8
A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8
A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10
A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1
A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12
A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2
A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14
A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
A8	sample8-rep1	3500 nt to 5389 nt	40	80	82.5	4079	16
A8	sample8-rep1	5389 nt to 13000 nt	4	8	10.5	6774	1.6
A9	sample9-rep1	3500 nt to 5389 nt	45	90	92.5	4053	18
A9	sample9-rep1	5389 nt to 13000 nt	4.5	9	11.5	6916	1.8
A10	sample10-rep1	3500 nt to 5389 nt	50	100	102.5	4045	20
A10	sample10-rep1	5389 nt to 13000 nt	5	10	12.5	6870	2
A11	sample11-rep1	3500 nt to 5389 nt	55	110	112.5	4089	22
A11	sample11-rep1	5389 nt to 13000 nt	5.5	11	13.5	7320	2.2
A12	sample12-rep1	3500 nt to 5389 nt	60	120	122.5	4061	24
A12	sample12-rep1	5389 nt to 13000 nt	6	12	14.5	7135	2.4
B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62
B8	sample8-rep2	3500 nt to 5389 nt	40.5	81	83.5	4091	16.2
B8	sample8-rep2	5389 nt to 13000 nt	4.55	9	11.6	5534	1.82
B9	sample9-rep2	3500 nt to 5389 nt	45.5	91	93.5	4061	18.2
B9	sample9-rep2	5389 nt to 13000 nt	5.05	10	12.6	4079	2.02
B10	sample10-rep2	3500 nt to 5389 nt	50.5	101	103.5	4033	20.2
B10	sample10-rep2	5389 nt to 13000 nt	5.55	11	13.6	6807	2.22
B11	sample11-rep2	3500 nt to 5389 nt	55.5	111	113.5	4069	22.2
B11	sample11-rep2	5389 nt to 13000 nt	6.05	12	14.6	7000	2.42
B12	sample12-rep2	3500 nt to 5389 nt	60.5	121	123.5	4067	24.2
B12	sample12-rep2	5389 nt to 13000 nt	6.55	13	15.6	7094	2.62
C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4
C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4
C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4
C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4
C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4
C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4
C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4
C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84
C8	sample8-rep3	3500 nt to 5389 nt	41	82	84.5	4089	16.4
C8	sample8-rep3	5389 nt to 13000 nt	5.1	10	12.7	5684	2.04
C9	sample9-rep3	3500 nt to 5389 nt	46	92	94.5	4065	18.4
C9	sample9-rep3	5389 nt to 13000 nt	5.6	11	13.7	5530	2.24
C10	sample10-rep3	3500 nt to 5389 nt	51	102	104.5	4037	20.4
C10	sample10-rep3	5389 nt to 13000 nt	6.1	12	14.7	6551	2.44
C11	sample11-rep3	3500 nt to 5389 nt	56	112	114.5	4061	22.4
C11	sample11-rep3	5389 nt to 13000 nt	6.6	13	15.7	6970	2.64
C12	sample12-rep3	3500 nt to 5389 nt	61	122	124.5	4070	24.4
C12	sample12-rep3	5389 nt to 13000 nt	7.1	14	16.7	6740	2.84
D1	sample13-rep1	3500 nt to 5389 nt	65	130	15.5	4079	2.6
D1	sample13-rep1	5389 nt to 13000 nt	2.15	13	6.8	4079	0.86
D2	sample13-rep2	3500 nt to 5389 nt	65.5	131	25.5	3757	4.6
D2	sample13-rep2	5389 nt to 13000 nt	2.65	14	7.8	9444	1.06
D3	sample13-rep3	3500 nt to 5389 nt	66	132	35.5	4079	6.6
D3	sample13-rep3	5389 nt to 13000 nt	3.15	15	8.8	4079	1.26
D4	sample14-rep1	3500 nt to 5389 nt	70	140	45.5	4079	8.6
D4	sample14-rep1	5389 nt to 13000 nt	3.65	14	9.8	4079	1.46
D5	sample14-rep2	3500 nt to 5389 nt	70.5	141	55.5	5026	10.6
D5	sample14-rep2	5389 nt to 13000 nt	4.15	15	10.8	6983	1.66
D6	sample14-rep3	3500 nt to 5389 nt	71	142	65.5	5240	12.6
D6	sample14-rep3	5389 nt to 13000 nt	4.65	16	11.8	6440	1.86
D7	sample15-rep1	3500 nt to 5389 nt	75	150	75.5	5240	14.6
D7	sample15-rep1	5389 nt to 13000 nt	5.15	15	12.8	6440	2.06
D8	sample15-rep2	3500 nt to 5389 nt	75.5	151	85.5	4079	16.6
D8	sample15-rep2	5389 nt to 13000 nt	5.65	16	13.8	4079	2.26
D9	sample15-rep3	3500 nt to 5389 nt	76	152	95.5	3757	18.6
D9	sample15-rep3	5389 nt to 13000 nt	6.15	17	14.8	9444	2.46
D10	Blank-rep1	3500 nt to 5389 nt	80	160	105.5	4079	20.6
D10	Blank-rep1	5389 nt to 13000 nt	6.65	16	15.8	4079	2.66
D11	Blank2-rep1	3500 nt to 5389 nt	80.5	161	115.5	4079	22.6
D11	Blank2-rep1	5389 nt to 13000 nt	7.15	17	16.8	4079	2.86
D12	Ladder	3500 nt to 5389 nt	81	162	125.5	5026	24.6
D12	Ladder	5389 nt to 13000 nt	7.65	18	17.8	6983	3.06

Pass/Fail Parameters  
547  
result >> 547

### RESULTS FOR VALIDATION DATA

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				
									Sample ID	Average	stdev	%CV	
	1 A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2	sample1-rep1	11.00	1.00	9.09	FAIL
	2 B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2					
	3 C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4					
	1 A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4	sample2-rep1	21.00	1.00	4.76	FAIL
	2 B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2					
	3 C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4					
	1 A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6	sample3-rep1	31.00	1.00	3.23	FAIL
	2 B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2					
	3 C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4					
	1 A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8	sample4-rep1	41.00	1.00	2.44	FAIL
	2 B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2					
	3 C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4					
	1 A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10	sample5-rep1	51.00	1.00	1.96	FAIL
	2 B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2					
	3 C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4					
	1 A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12	sample6-rep1	61.00	1.00	1.64	PASS
	2 B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2					
	3 C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4					
	1 A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14	sample7-rep1	71.00	1.00	1.41	PASS
	2 B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2					
	3 C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4					
	1 A8	sample8-rep1	3500 nt to 5389 nt	40	80	82.5	4079	16	sample8-rep1	81.00	1.00	1.23	PASS
	2 B8	sample8-rep2	3500 nt to 5389 nt	40.5	81	83.5	4091	16.2					
	3 C8	sample8-rep3	3500 nt to 5389 nt	41	82	84.5	4089	16.4					
	1 A9	sample9-rep1	3500 nt to 5389 nt	45	90	92.5	4053	18	sample9-rep1	91.00	1.00	1.10	PASS
	2 B9	sample9-rep2	3500 nt to 5389 nt	45.5	91	93.5	4061	18.2					
	3 C9	sample9-rep3	3500 nt to 5389 nt	46	92	94.5	4065	18.4					
	1 A10	sample10-rep1	3500 nt to 5389 nt	50	100	102.5	4045	20	sample10-rep1	101.00	1.00	0.99	PASS
	2 B10	sample10-rep2	3500 nt to 5389 nt	50.5	101	103.5	4033	20.2					
	3 C10	sample10-rep3	3500 nt to 5389 nt	51	102	104.5	4037	20.4					
	1 A11	sample11-rep1	3500 nt to 5389 nt	55	110	112.5	4089	22	sample11-rep1	111.00	1.00	0.90	PASS
	2 B11	sample11-rep2	3500 nt to 5389 nt	55.5	111	113.5	4069	22.2					
	3 C11	sample11-rep3	3500 nt to 5389 nt	56	112	114.5	4061	22.4					
	1 A12	sample12-rep1	3500 nt to 5389 nt	60	120	122.5	4061	24	sample12-rep1	121.00	1.00	0.83	PASS
	2 B12	sample12-rep2	3500 nt to 5389 nt	60.5	121	123.5	4067	24.2					
	3 C12	sample12-rep3	3500 nt to 5389 nt	61	122	124.5	4070	24.4					
	1 D1	sample13-rep1	3500 nt to 5389 nt	65	130	15.5	4079	2.6	sample13-rep1	131.00	1.00	0.76	PASS
	2 D2	sample13-rep2	3500 nt to 5389 nt	65.5	131	25.5	3757	4.6					
	3 D3	sample13-rep3	350										

VALIDATION DATA								
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62
3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84

RESULTS FOR VALIDATION DATA													
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				
									Sample ID	Average	stdev	%CV	Result
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2	sample1-rep1	11.0	1.0	9.1	FAIL
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2					
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4					
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4	sample2-rep1	21.0	1.0	4.8	FAIL
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2					
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4					
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6	sample3-rep1	31.0	1.0	3.2	FAIL
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2					
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4					
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8	sample4-rep1	41.0	1.0	2.4	FAIL
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2					
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4					
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10	sample5-rep1	51.0	1.0	2.0	FAIL
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2					
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4					
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12	sample6-rep1	61.0	1.0	1.6	PASS
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2					
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4					
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14	sample7-rep1	71.0	1.0	1.4	PASS
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2					
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4					

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	Result
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2	sample1-rep1	2.0	1.0	50.0	
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42					
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64					
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4	sample2-rep1	3.0	1.0	33.3	
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62					
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84					
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6	sample3-rep1	4.0	1.0	25.0	
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82					
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04					
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8	sample4-rep1	5.0	1.0	20.0	
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02					
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24					
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1	sample5-rep1	6.0	1.0	16.7	
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22					
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44					
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2	sample6-rep1	7.0	1.0	14.3	
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42					
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64					
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4	sample7-rep1	8.0	1.0	12.5	
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62					
3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84					

## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2021 11 01\14-29-08\2021 11 01 14H 29M.raw  
**Created:** Monday, November 1, 2021 2:55:05 PM  
**Number of capillaries:** 10  
**Array serial number:** 022621-27SFS  
**Effect length:** 33 cm  
**Array usage count:** 31  
**Instrument type:** 5300 Fragment Analyzer  
**Instrument controller software version:** 3.1.0.12  
**Device serial number:** MY2105AB19

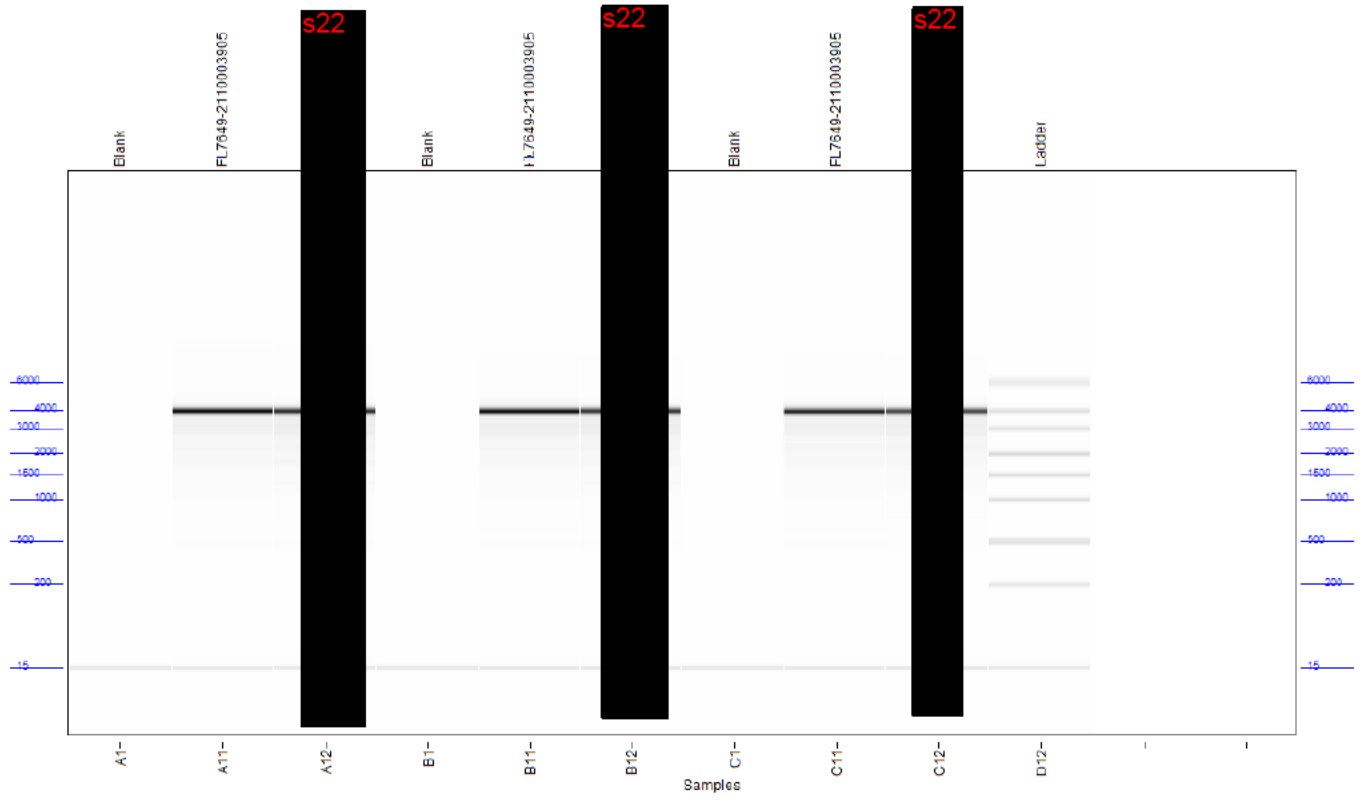
## Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds  
**Gel prime:** No  
**Full conditioning:** Yes  
**Gel prime to buffer:** Yes  
**Gel selection:** Gel 2  
**Perform prerun:** 8.0 kV, 30 sec.  
**Rinse:** No  
**Marker 1:** No  
**Rinse:** Tray: 3, Row: A, Dip count: 2  
**Sample injection:** 5.0 kV, 6 sec.  
**Separation:** 8.0 kV, 60.0 min.  
**Tray name:** Tray-1

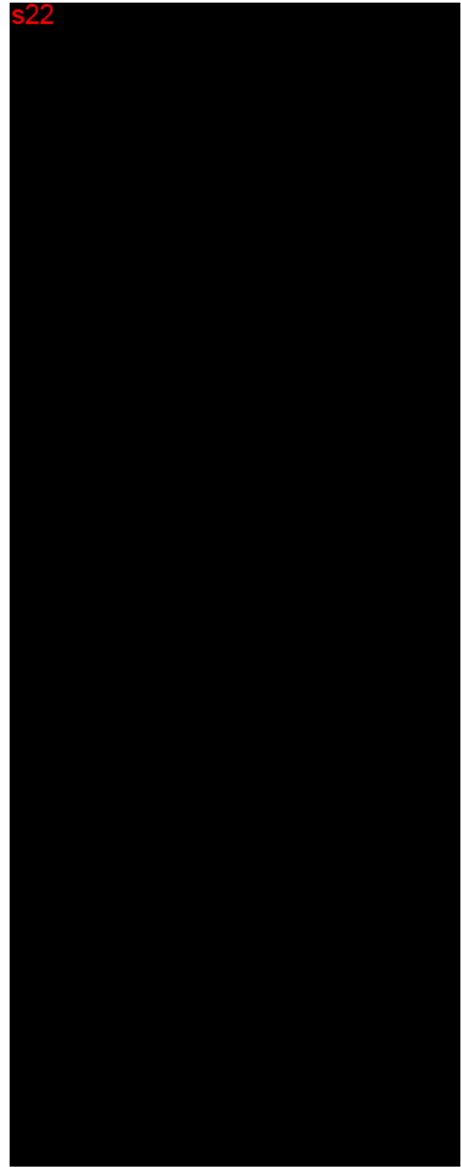
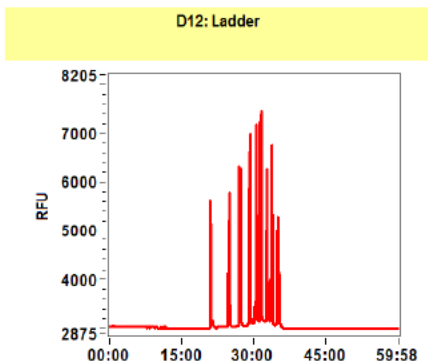
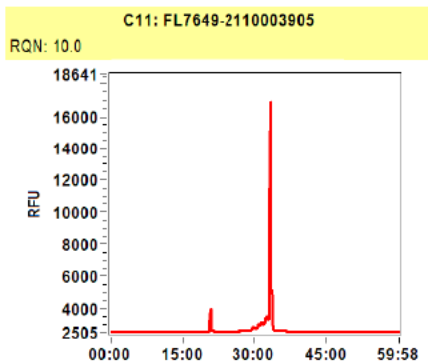
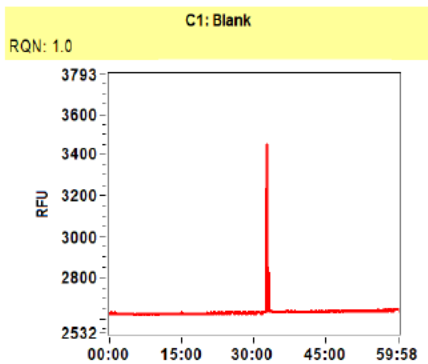
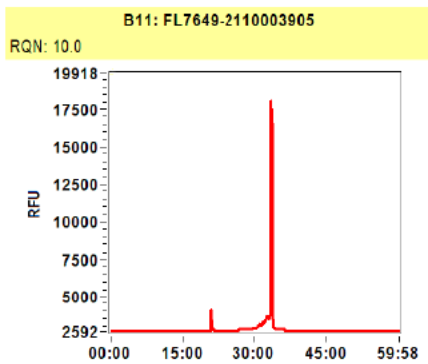
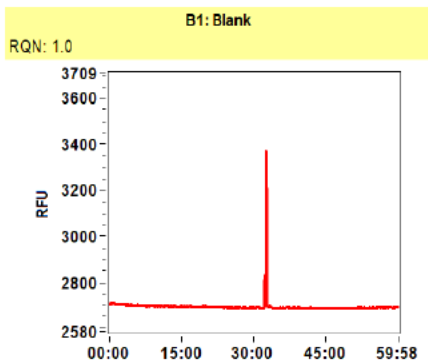
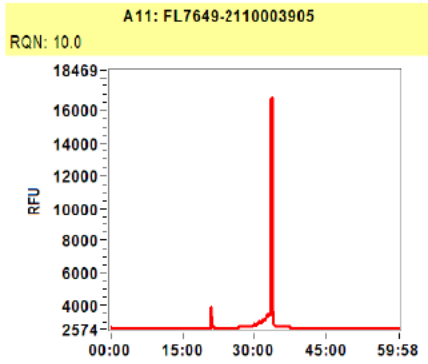
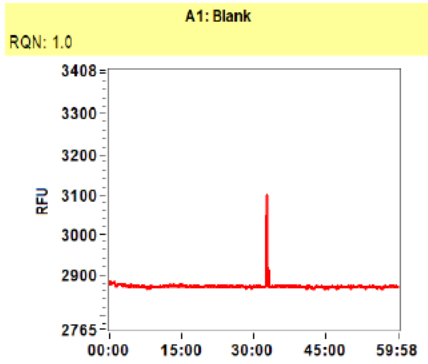
**Analysis mode:** RNA (Eukaryotic)

## Notes

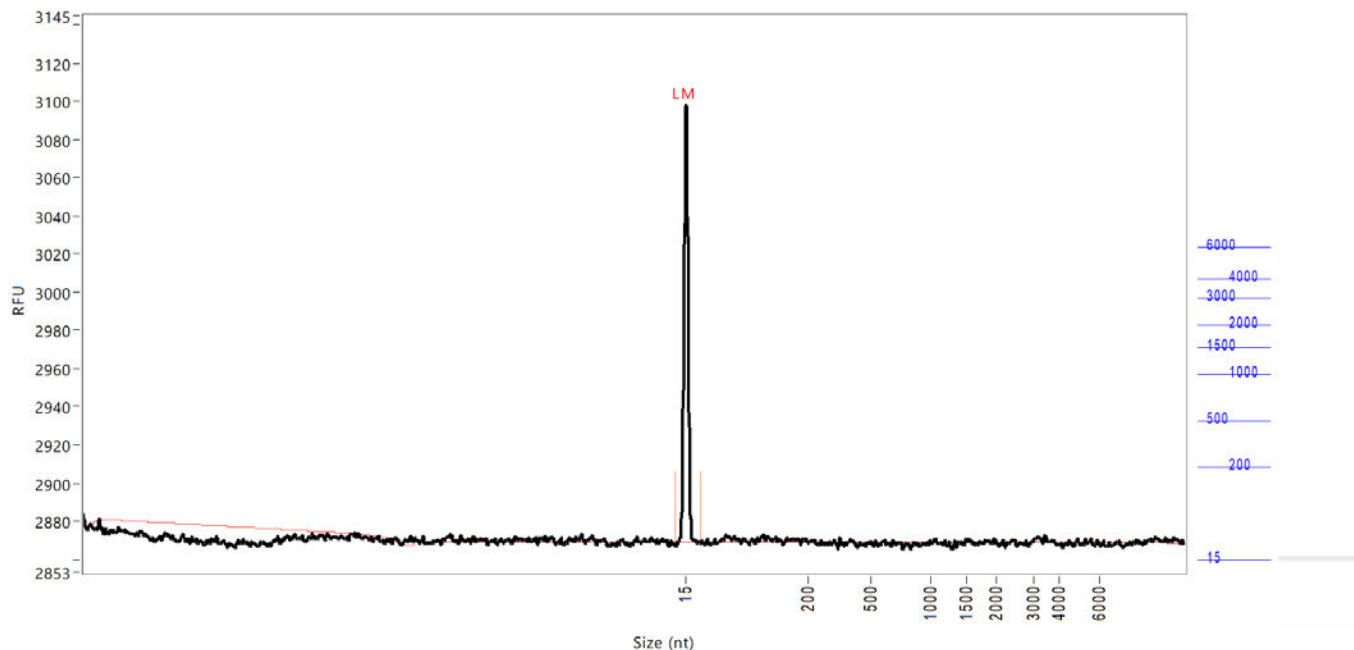
### Gel Image



s22



**Sample:** Blank  
**Well location:** A1  
**Created:** Monday, November 1, 2021 2:55:05 PM



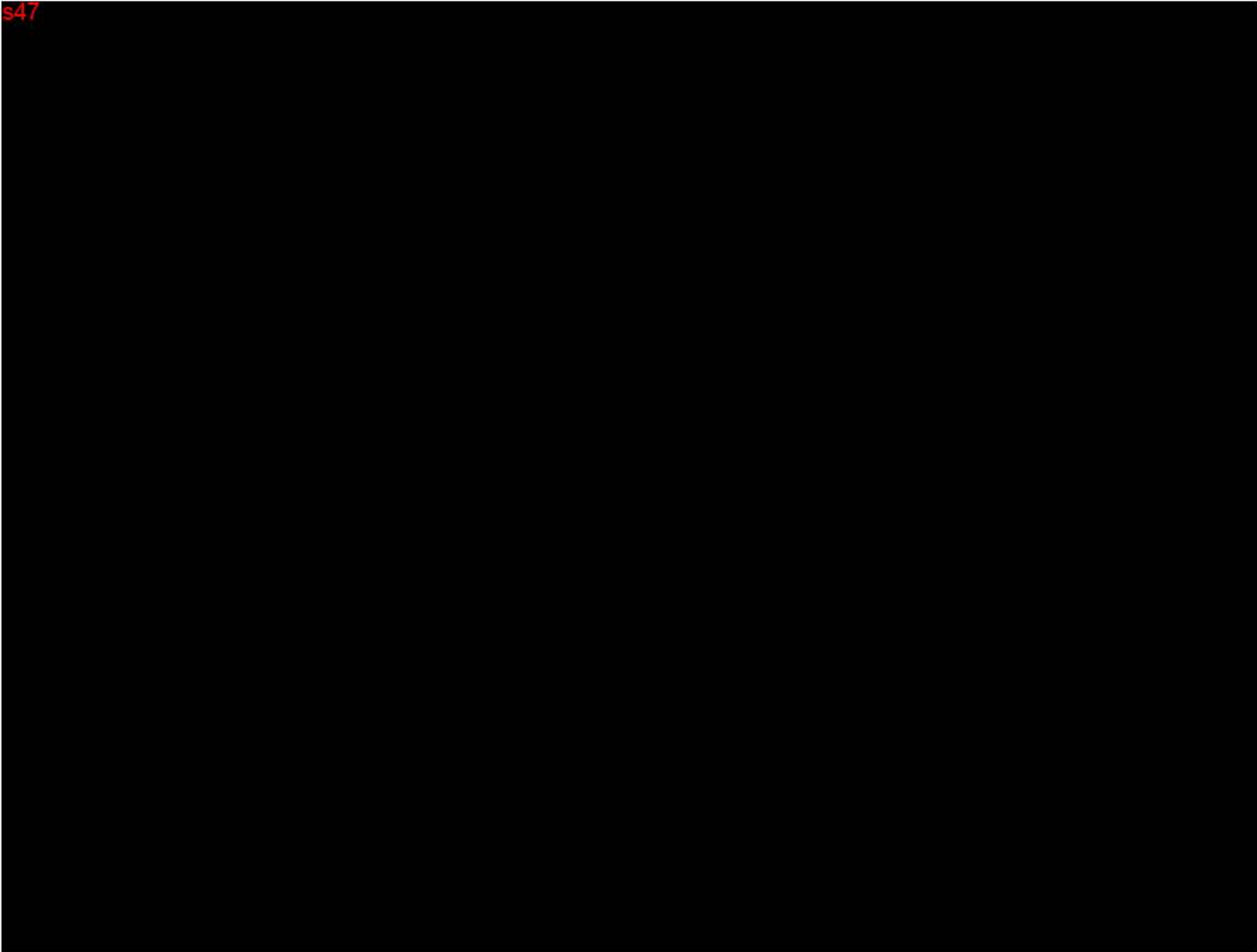
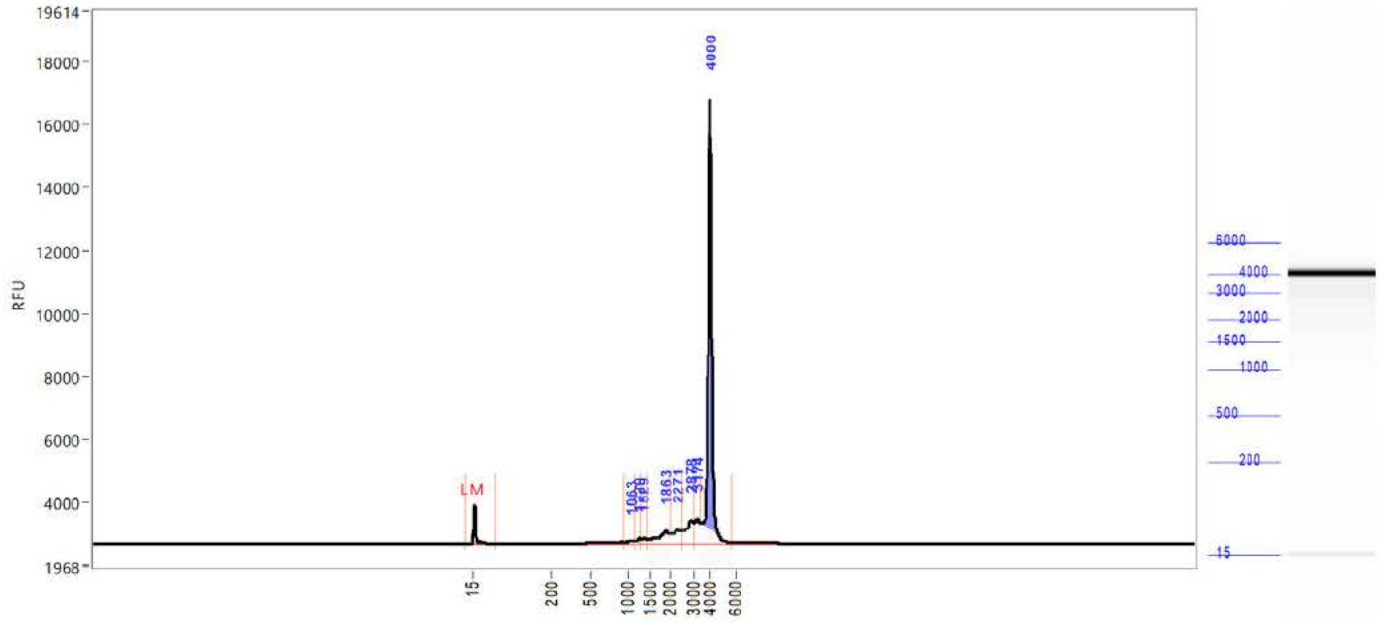
Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.6303	0	37	227
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0805	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0114 ng/ul	14.1 %Total	0.0037 nmole/L	9597 Avg. Size (nt)	3.84 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

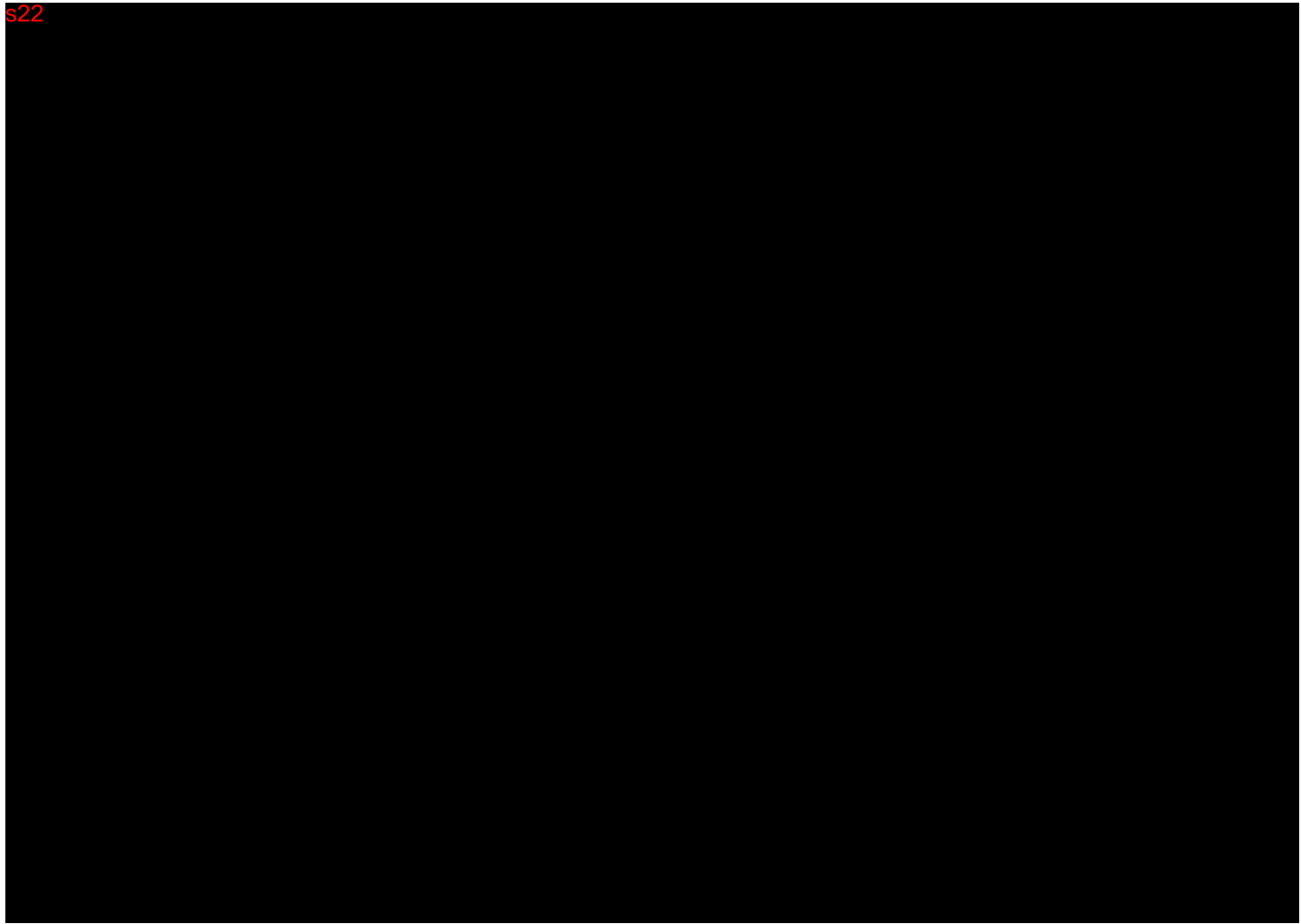


Sample: FL7649-2110003905

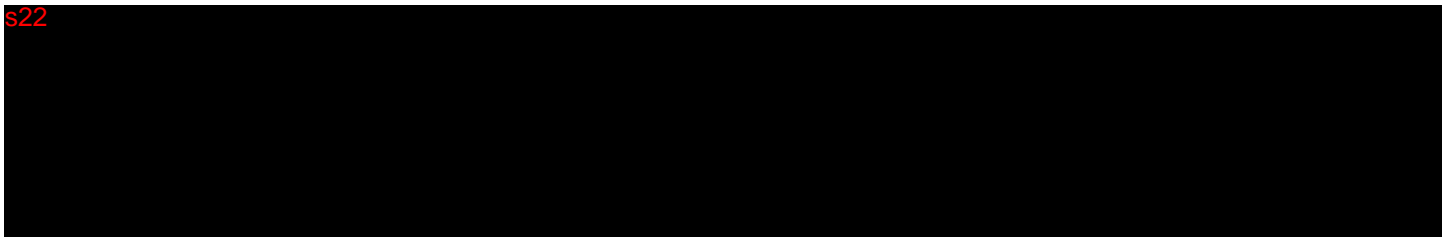


s22



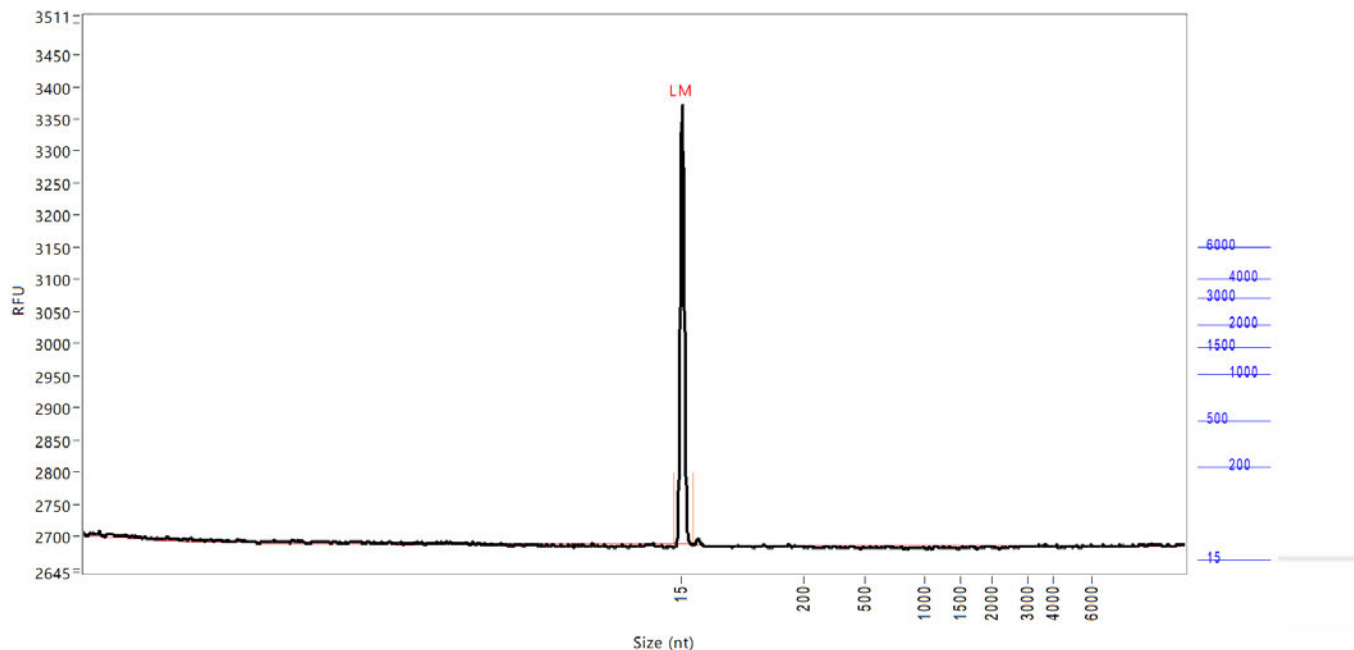


s22



s22

**Sample:** Blank  
**Well location:** B1  
**Created:** Monday, November 1, 2021 2:55:05 PM



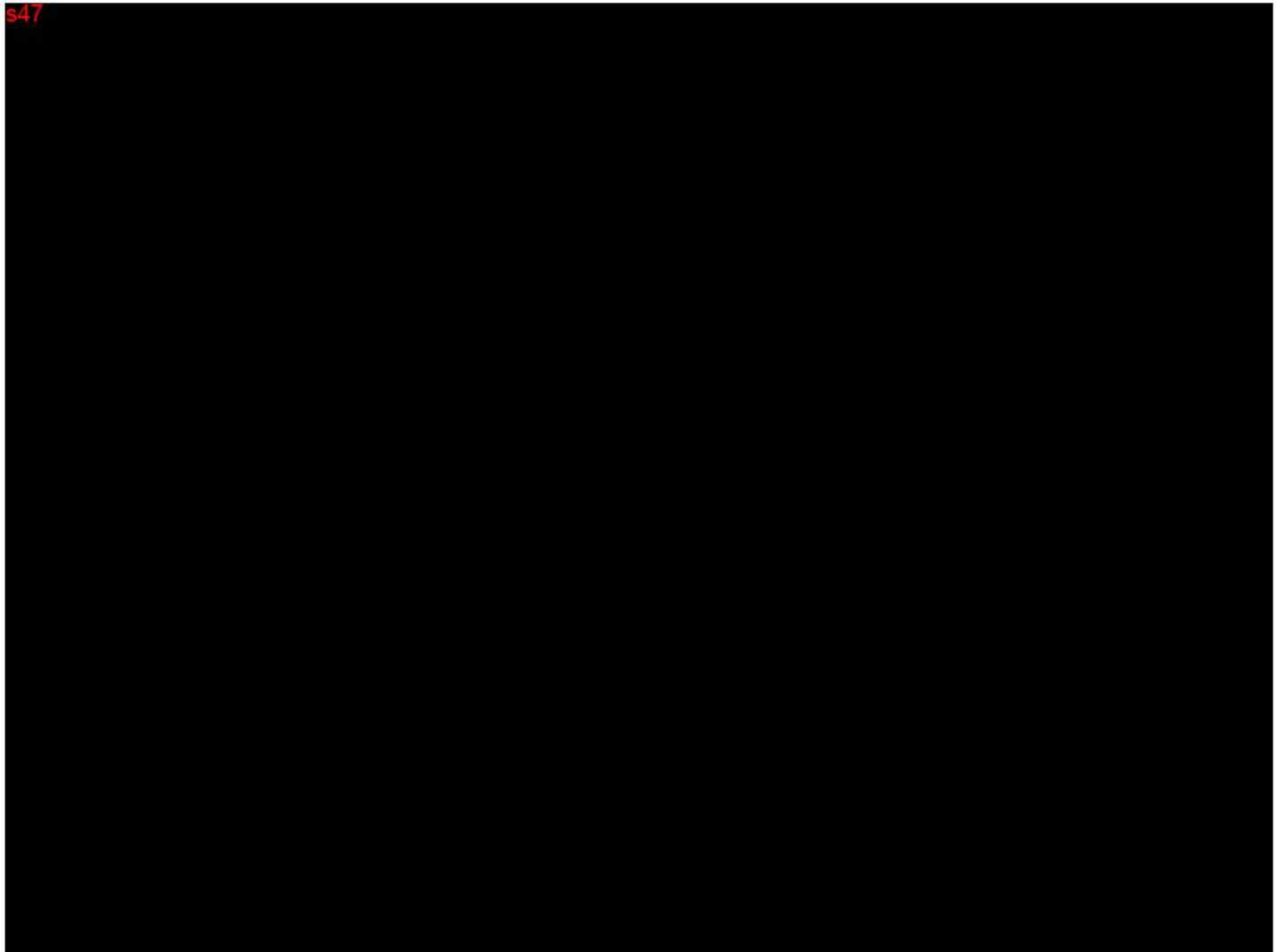
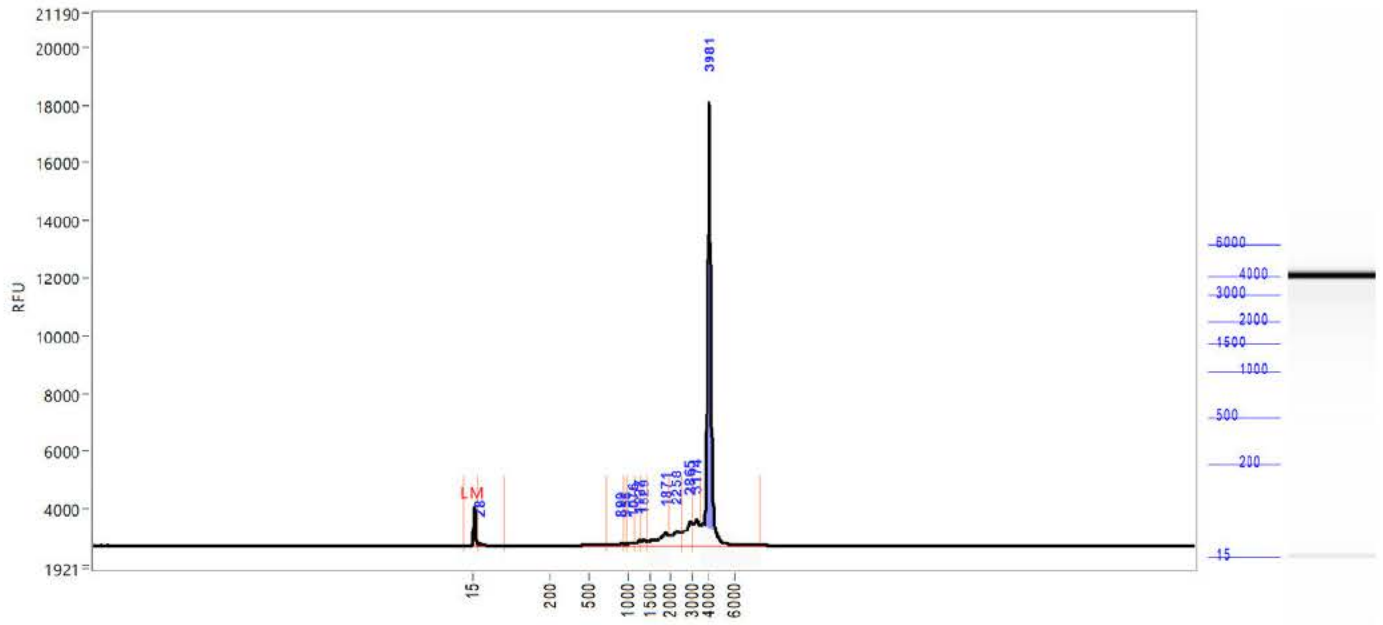
Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.6303	2	33	682
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0444	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0035 ng/ul	7.8 %Total	0.0012 nmole/L	9317 Avg. Size (nt)	4.66 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

Sample: FI 7649-2110003905

s22

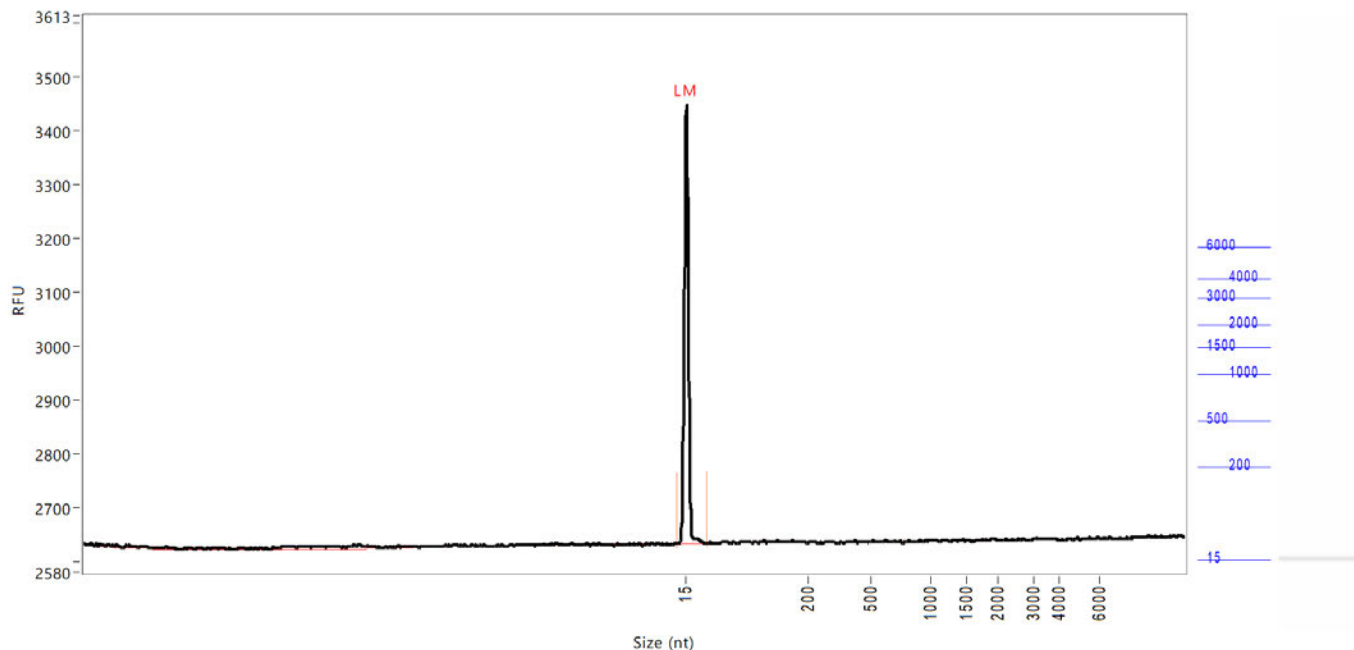


s47

S22



**Sample:** Blank  
**Well location:** C1  
**Created:** Monday, November 1, 2021 2:55:05 PM



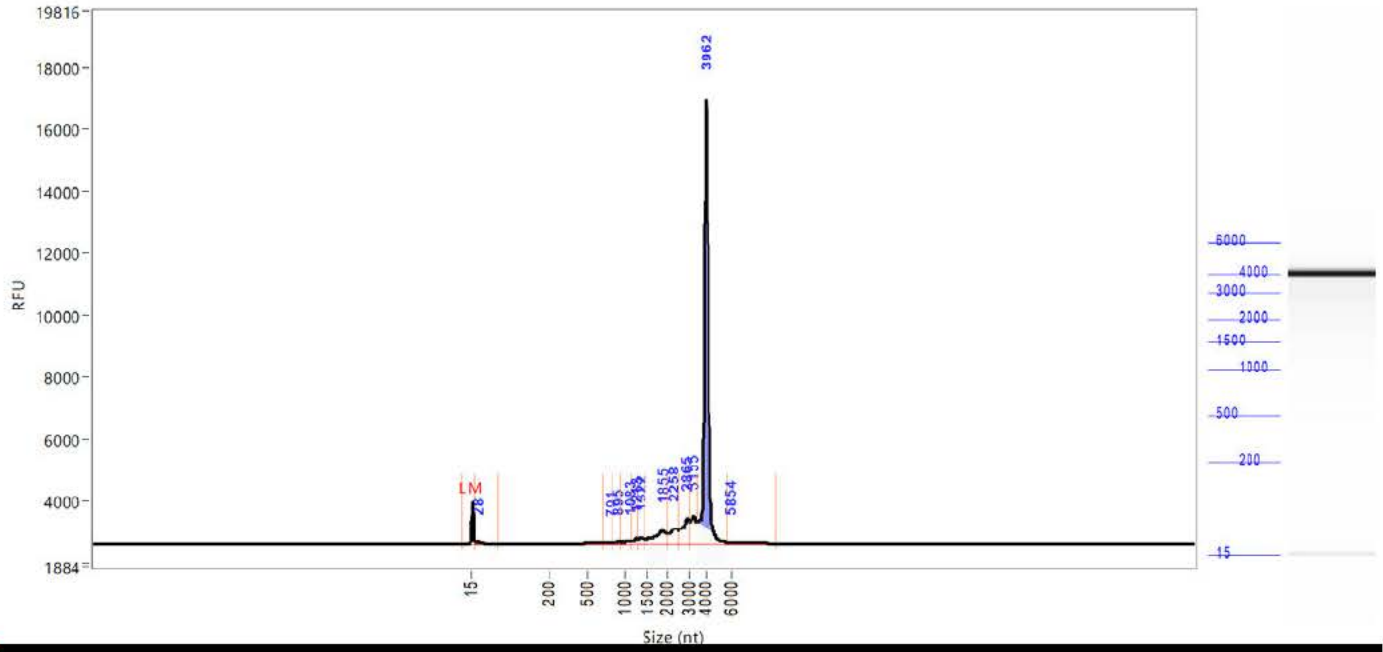
Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.6303	2	47	813
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0083	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/uL	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0065 ng/uL	79.0 %Total	0.0023 nmole/L	8752 Avg. Size (nt)	2.85 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

Sample: FL7649-2110003905

s22



s47



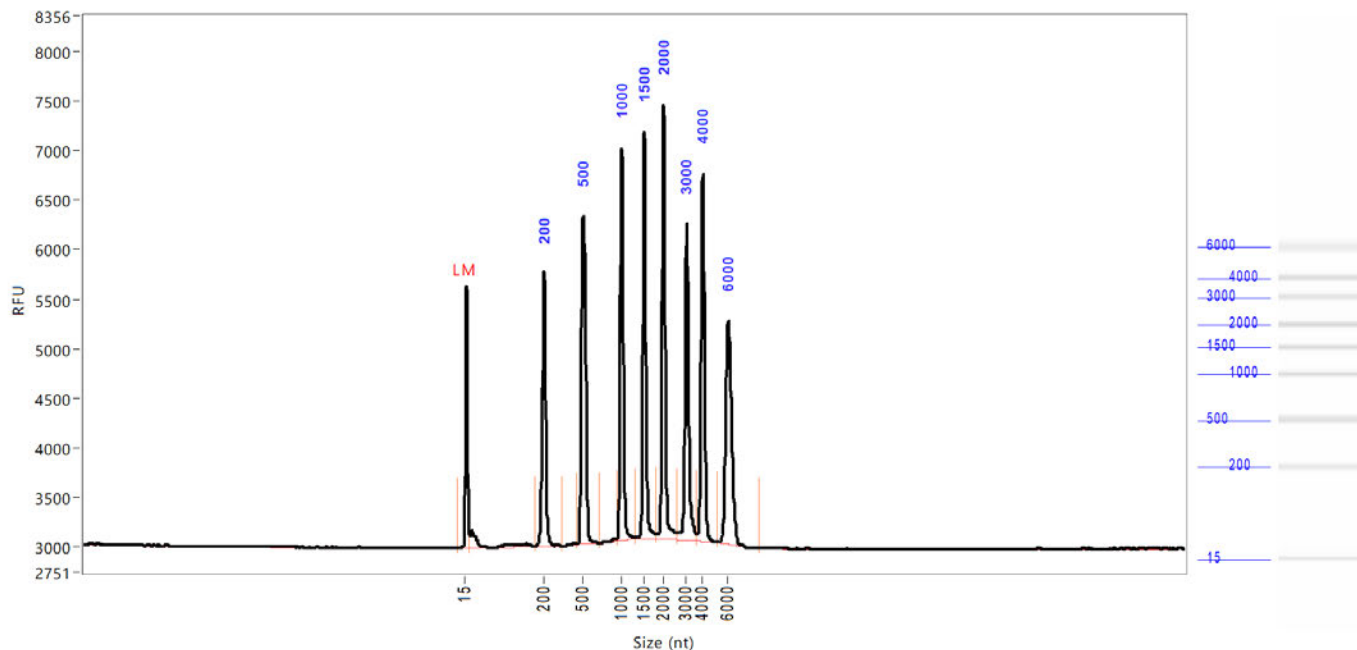
522



s22



**Sample:** Ladder  
**Well location:** D12  
**Created:** Monday, November 1, 2021 2:55:05 PM



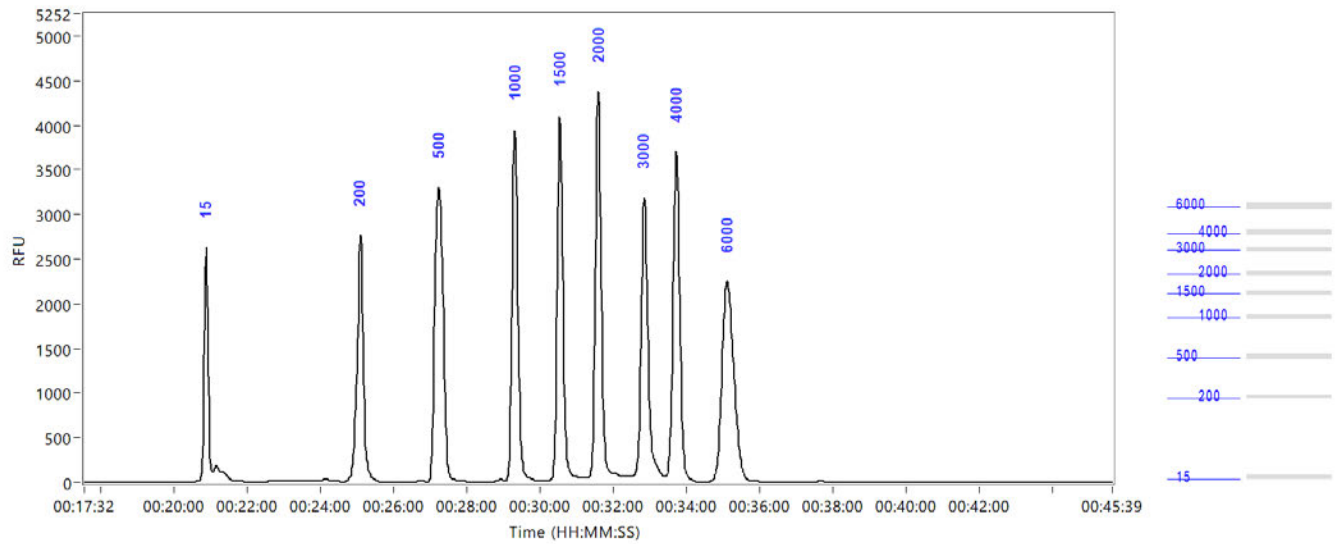
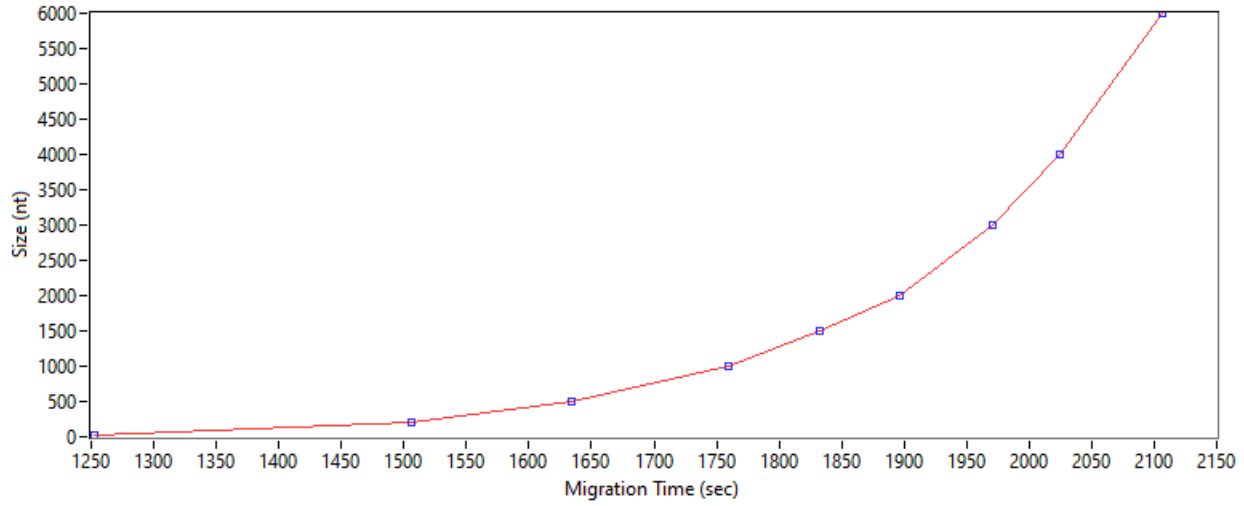
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.6303	0	23	2639
2	200	10.3806	179	342	2767
3	500	15.3432	455	718	3298
4	1000	11.7140	936	1309	3946
5	1500	11.5749	1309	1791	4099
6	2000	13.1244	1791	2595	4374
7	3000	10.7897	2595	3654	3192
8	4000	11.4257	3654	5147	3706
9	6000	11.5488	5147	8484	2252

TIC: 95.9013 ng/uL  
 TIM: 362.6375 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6      Sample min peak height: 200      Sample baseline V to V?: Y      Sample baseline V to V points: 3  
 Sample filter: Binomial      Number of points for filter: 9      Sample start region (min): 0      Sample end region (min): 60  
 Marker peak width (sec): 6      Marker min peak height: 100      Marker baseline V to V?: Y      Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU      Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder      Final concentration (ng/uL): 8.0000      Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Monday, November 1, 2021 2:55:05 PM  
**Fit type:** Point to point

Calibration curve





<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Appendix 1 - Fragment Analyzer Worksheet - Pfizer COMIRNATY	

## Worksheet for Fragment Analyzer - RNA Integrity

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-26	<b>Analysist</b>	s22
<b>TRIM link to data files</b>	D21-3280419, D21-3280418	<b>Test Date</b>	1/11/2021

Pipettes & Equipment	
Name	LIMS#
30-300 µL 12 channel pipette	N/A
p10 pipette	32835
p50 pipette	N/A
p100 pipette	32792
p200 pipette	5649
Thermomixer	23660
Thermocycler	N/A
P20	32891

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	N/A
Standard Sensitivity (SS) RNA kit Part 1 stored at 2-8°C	DNF-471-0500	0006609959	10/03/2022
<i>Extra Blank solution</i>	DNF-300-0008	6594431	3/03/2022
Standard Sensitivity (SS) RNA kit Part 2 stored at -20°C (Diluent Marker & Intercalating dye)	Enter text.	Enter text.	Enter a date.
<i>Extra Diluent marker</i>	DNF-369-0004	0006602443	7/04/2023
Standard Sensitivity (SS) RNA kit Part 3 stored at -70°C (RNA Ladder)	DNF-382-U020	0006600148	29/03/2022
Capillary conditioning solution	DNF-475-0100	6598614	22/03/2022
DEPC water	AM9961	2004017	N/A
20% Triton-X100 / 30% Ethanol solution	In house	MC1SEP21-01	1/02/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Intercalating dye	Dnf-600-u030	6603014	9/04/2022
Enter text.	Enter text.	Enter text.	Enter a date.

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT (Drawer B)	1/11/2021	2/11/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT (Drawer M)	1/11/2021	2/11/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT (Drawer 3)	26/10/2021	9/11/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	1/11/2021	2/11/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	1/11/2021	3/11/2021  48 hours
<b>Empty waste tray and waste bottle</b> <b>Reagents can be scaled up if required – this table provides the minimum for a single run.</b> <b>Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</b> <b>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</b>  <b>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</b>			

#### 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BF-25	BF-25	BF-25	BF-25	BF-25	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	BF-25	BF-25	BF-25	BF-25	BF-25	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	BF-25	BF-25	BF-25	BF-25	BF-25	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	L

**S1-6** = Samples in triplicate (a, b or c),

**RM** = Reference material

**BF-25** = Blank solution provided in kit,

**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test. Any samples not included in the test must be crossed off the plate layout, and results table below

System Suitability Criteria – RNA Ladder			
Plate location (wells)	D12		
Parameter	Limits	Results	Comments
RNA ladder profile	Visually comparable to figure 4 of SOP	ok	PASS
All peaks present	15 200 500 1000 1500 2000 3000 4000 6000 nt	ok	PASS
Peak heights	<60000 RFU	ok	PASS
Assay Acceptance Criteria – Reference Material			
Plate location (wells)	A12 B12 C12		
LIMS #	2108002914		
BATCH #	EE8493		
EXPIRY	5/02/2022		
Parameter	Limits	Results	Comments
Profile	Visually comparable to DP electropherogram in SOP	Ok/ok/ok	PASS
Migration time	Approximately comparable to profile in SOP	3981/4000/3981	PASS
Lower marker present	LM peak	Ok/ok/ok	PASS
Peak heights	5000-600000 for 2/3 replicates	11936/11270/10990	PASS
No negative peaks or baseline shifts	No significant peaks/shifts	Ok/ok/ok	PASS
Reference Material Dilutions / Calculation / Notes			
thaw date: 19/10/21 270ng/uL = 20 uL of 530 ng/uL master stock + 19 uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution  The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919			

Sample 1 Details	
Plate location (wells)	A11 B11 C11
LIMS #	2110003905
BATCH #	FL7649
EXPIRY	28/02/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4000/3981/3962	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	14106/15399/14335	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>opened for the first time 01/11/21, stored at 2-8C            270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water            90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919</p>

Sample Results	
PASS	
Analysist	s22
Checked by	s22



Sample 2 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.
Sample 3 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	PASS
Lower marker	LM must be present	Enter text.	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>Thaw date – 25/10/2021 – stored cell culture fridge , opened for the first time 25/10/21, stored at 2-8C</p> <p>60 ng/uL = 20 uL of 200 ng/uL DP + 40 uL %Tx100 solution/30% ethanol</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919</p>

Sample Results	
<b>Choose an item.</b>	
Analysist	s22
Checked by	Enter text.
Sample 4 Details	
Plate location (wells)	A8 B8 C8
LIMS #	2110003932
BATCH #	s22
EXPIRY	5/05/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	3981/3981/3942	Choose an item.
Lower marker	LM must be present	Ok/ok/ok	Choose an item.

Peak heights	5000-60000 RFU for 2/3 replicates	13683/11870/13713	Choose an item.
--------------	--------------------------------------	-------------------	-----------------

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

Sample 5 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	Enter text.	Enter text.	Enter text.	Choose an item.

<b>% Late Migrating Species</b>		Enter text.	Enter text.	Enter text.	
---------------------------------	--	-------------	-------------	-------------	--

Sample Dilutions / Calculation / Notes	
Enter text.	

Sample Results	
----------------	--

<b>Choose an item.</b>	
<b>Analysist</b>	Enter text.
<b>Checked by</b>	Enter text.

Sample 6 Details	
------------------	--

<b>Plate location (wells)</b>	Choose an item.
<b>LIMS #</b>	<b>Click or tap here to enter text.</b>
<b>BATCH #</b>	Click or tap here to enter text.
<b>EXPIRY</b>	Enter date.

Acceptance Criteria			
---------------------	--	--	--

Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
--------------	--	--	--	--	--

Parameters	Limits	Results			Comments
		Average	SD	%RSD	
<b>% RNA Integrity</b>	s47	Enter text.	Enter text.	Enter text.	Choose an item.
<b>% Late Migrating Species</b>		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes	
Enter text.	

Sample Results	
----------------	--

<b>Choose an item.</b>	
------------------------	--

<b>Analysist</b>	
<b>Checked by</b>	Enter text.

<b>Notes</b>
Enter text.





Owner: s22	Number: Bio-BEE-Form-42
Author: s22	Version: 1
Active: 2/07/2021	Review: <QPulse_DocReviewDate>
Title: Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 28Oct2021/2

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899LRW Expiry: 18 March 2022Other Reagent: Pyrospense Batch# 0000904583  
November 2021Expiry: 29 June 2022 Use By: 19

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Pfizer Comirnaty</u>	<u>FL7649</u>	<u>28 February 2022</u>	<u>2110003905-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
<u>n/a</u>	s47	<u>2500</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of vaccine</u>	<u>975uL</u>	<u>5uL pyrospense</u>
<u>1/20</u>	<u>50uL of 1/50 dilution</u>	<u>945uL</u>	<u>5uL pyrospense</u>
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>N/A (Undefined)</u>	s47	<u>0.24</u>	<u>164</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

**Notes:**

**Checked** s22 28Oct2021



Data from Smear Analysis Table

1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate
2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the avg smear sets.
3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	A1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A1	A1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A2	A2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A2	A2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A3	A3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A3	A3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A4	A4	Blank	3700 nt to 4800 nt	0.0052	1.5	0.0042	3891	1.86
A4	A4	Blank	4800 nt to 13000 nt	0.0848	24.3	0.0266	9946	21.16
A5	A5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A5	A5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A6	A6	Blank	3700 nt to 4800 nt	0.0012	2.7	0.0009	4289	0.27
A6	A6	Blank	4800 nt to 13000 nt	0.0004	0.9	0.0002	7702	0
A7	A7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	NaN	NaN
A7	A7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
A8	A8	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3790	1.66
A8	A8	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
A9	A9	FG3712 - 210900						
A9	A9	FG3712 - 210900						
§22								
B1	B1	Blank	3700 nt to 4800 nt	0.0137	100	0.0102	4188	2.42
B1	B1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B2	B2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B2	B2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B3	B3	Blank	3700 nt to 4800 nt	0.023	65.4	0.0177	4048	2.82
B3	B3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B4	B4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B4	B4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B5	B5	Blank	3700 nt to 4800 nt	0.0003	0.9	0.0002	3898	0.44
B5	B5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B6	B6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B6	B6	Blank	4800 nt to 13000 nt	0.0018	100	0.0007	7459	0.82
B7	B7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3895	6.13
B7	B7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	9158	25.66
B8	B8	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3823	2.27
B8	B8	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	9213	21.12
B9	B9	FG3712 - 210900						
B9	B9	FG3712 - 210900						
§22								
C1	C1	Blank	3700 nt to 4800 nt	0.0152	60.5	0.0118	3995	2.29
C1	C1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C2	C2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C2	C2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C3	C3	Blank	3700 nt to 4800 nt	0.0012	0.9	0.0009	3947	1.23
C3	C3	Blank	4800 nt to 13000 nt	0.1246	95.7	0.0422	9205	14.48
C4	C4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C4	C4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C5	C5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C5	C5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C6	C6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C6	C6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C7	C7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3753	0.99
C7	C7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
C8	C8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C8	C8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C9	C9	FG3712 - 210900						
C9	C9	FG3712 - 210900						
§22								
D1	D1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3772	1.38
D2	D2	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
D3	D3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	4800 nt to 13000 nt	0.0013	100	0.0006	7444	3.29
D4	D4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	4800 nt to 13000 nt	0.0061	99.9	0.0027	7118	0.49
D5	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	4800 nt to 13000 nt	0.0048	100	0.0021	7035	0.51
D6	D6	Blank	3700 nt to 4800 nt	0.0015	17.3	0.0013	3714	0.25
D6	D6	Blank	4800 nt to 13000 nt	0.0073	82.7	0.0036	6325	3.79
D7	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	4800 nt to 13000 nt	0.0031	100	0.0013	7176	3.63
D8	D8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D9	D9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D9	D9	Blank	4800 nt to 13000 nt	0.0066	33.7	0.0038	5389	1.29
D10	D10	Blank	3700 nt to 4800 nt	0.1382	1.5	0.0998	4321	6.06
D10	D10	Blank	4800 nt to 13000 nt	0.0729	0.8	0.0435	5230	8.57
D11	D11	Blank	3700 nt to 4800 nt	0.039	0.9	0.0299	4075	10.09
D11	D11	Blank	4800 nt to 13000 nt	0.0228	0.5	0.0133	5348	12.2
D12	D12	Ladder						



[Redacted]										[Redacted]			
3	C9	FG3712 - 2109003215	[Redacted]							[Redacted]			
1	D1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	50.00	70.71	141.42	[Redacted]
2	D2	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN	Blank	50.00	70.71	141.42	[Redacted]
3	D3	Blank	4800 nt to 13000 nt	0.0013	100	0.0006	7444	3.29	Blank	50.00	70.71	141.42	[Redacted]
1	D4	Blank	4800 nt to 13000 nt	0.0061	99.9	0.0027	7118	0.49	Blank	94.20	9.96	10.57	[Redacted]
2	D5	Blank	4800 nt to 13000 nt	0.0048	100	0.0021	7035	0.51	Blank	94.20	9.96	10.57	[Redacted]
3	D6	Blank	4800 nt to 13000 nt	0.0073	82.7	0.0036	6325	3.79	Blank	94.20	9.96	10.57	[Redacted]
1	D7	Blank	4800 nt to 13000 nt	0.0031	100	0.0013	7176	3.63	Blank	66.85	46.88	70.13	[Redacted]
2	D8	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN	Blank	66.85	46.88	70.13	[Redacted]
3	D9	Blank	4800 nt to 13000 nt	0.0066	33.7	0.0038	5389	1.29	Blank	66.85	46.88	70.13	[Redacted]

This tab is only to be used if a replace needs to be excluded from the analysis.

Enter raw data directly into this table. All averages/ pass fail will be calculated automatically using the pass/fail parameters entered in the Calculations tab.

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS	
									Sample ID	Average	stdev	%CV		
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

Pass/Fail Parameters		
minimum	cut off	maximum
S47		
result >>	S47	

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
									Sample ID	Average	stdev	%CV	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	

VALIDATION DATA

Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2
A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4
A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4
A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6
A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8
A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8
A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10
A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1
A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12
A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2
A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14
A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
A8	sample8-rep1	3500 nt to 5389 nt	40	80	82.5	4079	16
A8	sample8-rep1	5389 nt to 13000 nt	4	8	10.5	6774	1.6
A9	sample9-rep1	3500 nt to 5389 nt	45	90	92.5	4053	18
A9	sample9-rep1	5389 nt to 13000 nt	4.5	9	11.5	6916	1.8
A10	sample10-rep1	3500 nt to 5389 nt	50	100	102.5	4045	20
A10	sample10-rep1	5389 nt to 13000 nt	5	10	12.5	6870	2
A11	sample11-rep1	3500 nt to 5389 nt	55	110	112.5	4089	22
A11	sample11-rep1	5389 nt to 13000 nt	5.5	11	13.5	7320	2.2
A12	sample12-rep1	3500 nt to 5389 nt	60	120	122.5	4061	24
A12	sample12-rep1	5389 nt to 13000 nt	6	12	14.5	7135	2.4
B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62
B8	sample8-rep2	3500 nt to 5389 nt	40.5	81	83.5	4091	16.2
B8	sample8-rep2	5389 nt to 13000 nt	4.55	9	11.6	5534	1.82
B9	sample9-rep2	3500 nt to 5389 nt	45.5	91	93.5	4061	18.2
B9	sample9-rep2	5389 nt to 13000 nt	5.05	10	12.6	4079	2.02
B10	sample10-rep2	3500 nt to 5389 nt	50.5	101	103.5	4033	20.2
B10	sample10-rep2	5389 nt to 13000 nt	5.55	11	13.6	6807	2.22
B11	sample11-rep2	3500 nt to 5389 nt	55.5	111	113.5	4069	22.2
B11	sample11-rep2	5389 nt to 13000 nt	6.05	12	14.6	7000	2.42
B12	sample12-rep2	3500 nt to 5389 nt	60.5	121	123.5	4067	24.2
B12	sample12-rep2	5389 nt to 13000 nt	6.55	13	15.6	7094	2.62
C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4
C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4
C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4
C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4
C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4
C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4
C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4
C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84
C8	sample8-rep3	3500 nt to 5389 nt	41	82	84.5	4089	16.4
C8	sample8-rep3	5389 nt to 13000 nt	5.1	10	12.7	5684	2.04
C9	sample9-rep3	3500 nt to 5389 nt	46	92	94.5	4065	18.4
C9	sample9-rep3	5389 nt to 13000 nt	5.6	11	13.7	5530	2.24
C10	sample10-rep3	3500 nt to 5389 nt	51	102	104.5	4037	20.4
C10	sample10-rep3	5389 nt to 13000 nt	6.1	12	14.7	6551	2.44
C11	sample11-rep3	3500 nt to 5389 nt	56	112	114.5	4061	22.4
C11	sample11-rep3	5389 nt to 13000 nt	6.6	13	15.7	6970	2.64
C12	sample12-rep3	3500 nt to 5389 nt	61	122	124.5	4070	24.4
C12	sample12-rep3	5389 nt to 13000 nt	7.1	14	16.7	6740	2.84
D1	sample13-rep1	3500 nt to 5389 nt	65	130	15.5	4079	2.6
D1	sample13-rep1	5389 nt to 13000 nt	2.15	13	6.8	4079	0.86
D2	sample13-rep2	3500 nt to 5389 nt	65.5	131	25.5	3757	4.6
D2	sample13-rep2	5389 nt to 13000 nt	2.65	14	7.8	9444	1.06
D3	sample13-rep3	3500 nt to 5389 nt	66	132	35.5	4079	6.6
D3	sample13-rep3	5389 nt to 13000 nt	3.15	15	8.8	4079	1.26
D4	sample14-rep1	3500 nt to 5389 nt	70	140	45.5	4079	8.6
D4	sample14-rep1	5389 nt to 13000 nt	3.65	14	9.8	4079	1.46
D5	sample14-rep2	3500 nt to 5389 nt	70.5	141	55.5	5026	10.6
D5	sample14-rep2	5389 nt to 13000 nt	4.15	15	10.8	6983	1.66
D6	sample14-rep3	3500 nt to 5389 nt	71	142	65.5	5240	12.6
D6	sample14-rep3	5389 nt to 13000 nt	4.65	16	11.8	6440	1.86
D7	sample15-rep1	3500 nt to 5389 nt	75	150	75.5	5240	14.6
D7	sample15-rep1	5389 nt to 13000 nt	5.15	15	12.8	6440	2.06
D8	sample15-rep2	3500 nt to 5389 nt	75.5	151	85.5	4079	16.6
D8	sample15-rep2	5389 nt to 13000 nt	5.65	16	13.8	4079	2.26
D9	sample15-rep3	3500 nt to 5389 nt	76	152	95.5	3757	18.6
D9	sample15-rep3	5389 nt to 13000 nt	6.15	17	14.8	9444	2.46
D10	Blank-rep1	3500 nt to 5389 nt	80	160	105.5	4079	20.6
D10	Blank-rep1	5389 nt to 13000 nt	6.65	16	15.8	4079	2.66
D11	Blank2-rep1	3500 nt to 5389 nt	80.5	161	115.5	4079	22.6
D11	Blank2-rep1	5389 nt to 13000 nt	7.15	17	16.8	4079	2.86
D12	Ladder	3500 nt to 5389 nt	81	162	125.5	5026	24.6
D12	Ladder	5389 nt to 13000 nt	7.65	18	17.8	6983	3.06

Pass/Fail Parameters
minimum    cut off    maximum
547
result >> 547

RESULTS FOR VALIDATION DATA

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				
									Sample ID	Average	stdev	%CV	
	1 A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2	sample1-rep1	11.00	1.00	9.09	FAIL
	2 B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2					
	3 C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4					
	1 A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4	sample2-rep1	21.00	1.00	4.76	FAIL
	2 B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2					
	3 C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4					
	1 A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6	sample3-rep1	31.00	1.00	3.23	FAIL
	2 B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2					
	3 C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4					
	1 A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8	sample4-rep1	41.00	1.00	2.44	FAIL
	2 B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2					
	3 C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4					
	1 A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10	sample5-rep1	51.00	1.00	1.96	FAIL
	2 B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2					
	3 C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4					
	1 A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12	sample6-rep1	61.00	1.00	1.64	PASS
	2 B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2					
	3 C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4					
	1 A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14	sample7-rep1	71.00	1.00	1.41	PASS
	2 B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2					
	3 C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4					
	1 A8	sample8-rep1	3500 nt to 5389 nt	40	80	82.5	4079	16	sample8-rep1	81.00	1.00	1.23	PASS
	2 B8	sample8-rep2	3500 nt to 5389 nt	40.5	81	83.5	4091	16.2					
	3 C8	sample8-rep3	3500 nt to 5389 nt	41	82	84.5	4089	16.4					
	1 A9	sample9-rep1	3500 nt to 5389 nt	45	90	92.5	4053	18	sample9-rep1	91.00	1.00	1.10	PASS
	2 B9	sample9-rep2	3500 nt to 5389 nt	45.5	91	93.5	4061	18.2					
	3 C9	sample9-rep3	3500 nt to 5389 nt	46	92	94.5	4065	18.4					
	1 A10	sample10-rep1	3500 nt to 5389 nt	50	100	102.5	4045	20	sample10-rep1	101.00	1.00	0.99	PASS
	2 B10	sample10-rep2	3500 nt to 5389 nt	50.5	101	103.5	4033	20.2					
	3 C10	sample10-rep3	3500 nt to 5389 nt	51	102	104.5	4037	20.4					
	1 A11	sample11-rep1	3500 nt to 5389 nt	55	110	112.5	4089	22	sample11-rep1	111.00	1.00	0.90	PASS
	2 B11	sample11-rep2	3500 nt to 5389 nt	55.5	111	113.5	4069	22.2					
	3 C11	sample11-rep3	3500 nt to 5389 nt	56	112	114.5	4061	22.4					
	1 A12	sample12-rep1	3500 nt to 5389 nt	60	120	122.5	4061	24	sample12-rep1	121.00	1.00	0.83	PASS
	2 B12	sample12-rep2	3500 nt to 5389 nt	60.5	121	123.5	4067	24.2					
	3 C12	sample12-rep3	3500 nt to 5389 nt	61	122	124.5	4070	24.4					
	1 D1	sample13-rep1	3500 nt to 5389 nt	65									

VALIDATION DATA								
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62
3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84

RESULTS FOR VALIDATION DATA										% INTEGRITY SUMMARY			
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2	sample1-rep1	11.0	1.0	9.1	FAIL
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2					
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4					
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4	sample2-rep1	21.0	1.0	4.8	FAIL
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2					
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4					
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6	sample3-rep1	31.0	1.0	3.2	FAIL
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2					
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4					
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8	sample4-rep1	41.0	1.0	2.4	FAIL
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2					
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4					
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10	sample5-rep1	51.0	1.0	2.0	FAIL
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2					
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4					
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12	sample6-rep1	61.0	1.0	1.6	PASS
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2					
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4					
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14	sample7-rep1	71.0	1.0	1.4	PASS
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2					
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4					

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2	sample1-rep1	2.0	1.0	50.0	
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42					
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64					
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4	sample2-rep1	3.0	1.0	33.3	
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62					
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84					
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6	sample3-rep1	4.0	1.0	25.0	
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82					
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04					
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8	sample4-rep1	5.0	1.0	20.0	
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02					
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24					
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1	sample5-rep1	6.0	1.0	16.7	
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22					
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44					
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2	sample6-rep1	7.0	1.0	14.3	
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42					
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64					
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4	sample7-rep1	8.0	1.0	12.5	
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62					
3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84					

## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2021 09 14\10-56-35\2021 09 14 10H 56M.raw  
**Created:** Tuesday, September 14, 2021 11:22:19 AM  
**Number of capillaries:** 16  
**Array serial number:** 022621-27SFS  
**Effect length:** 33 cm  
**Array usage count:** 23  
**Instrument type:** 5300 Fragment Analyzer  
**Instrument controller software version:** 3.1.0.12  
**Device serial number:** MY2105AB19

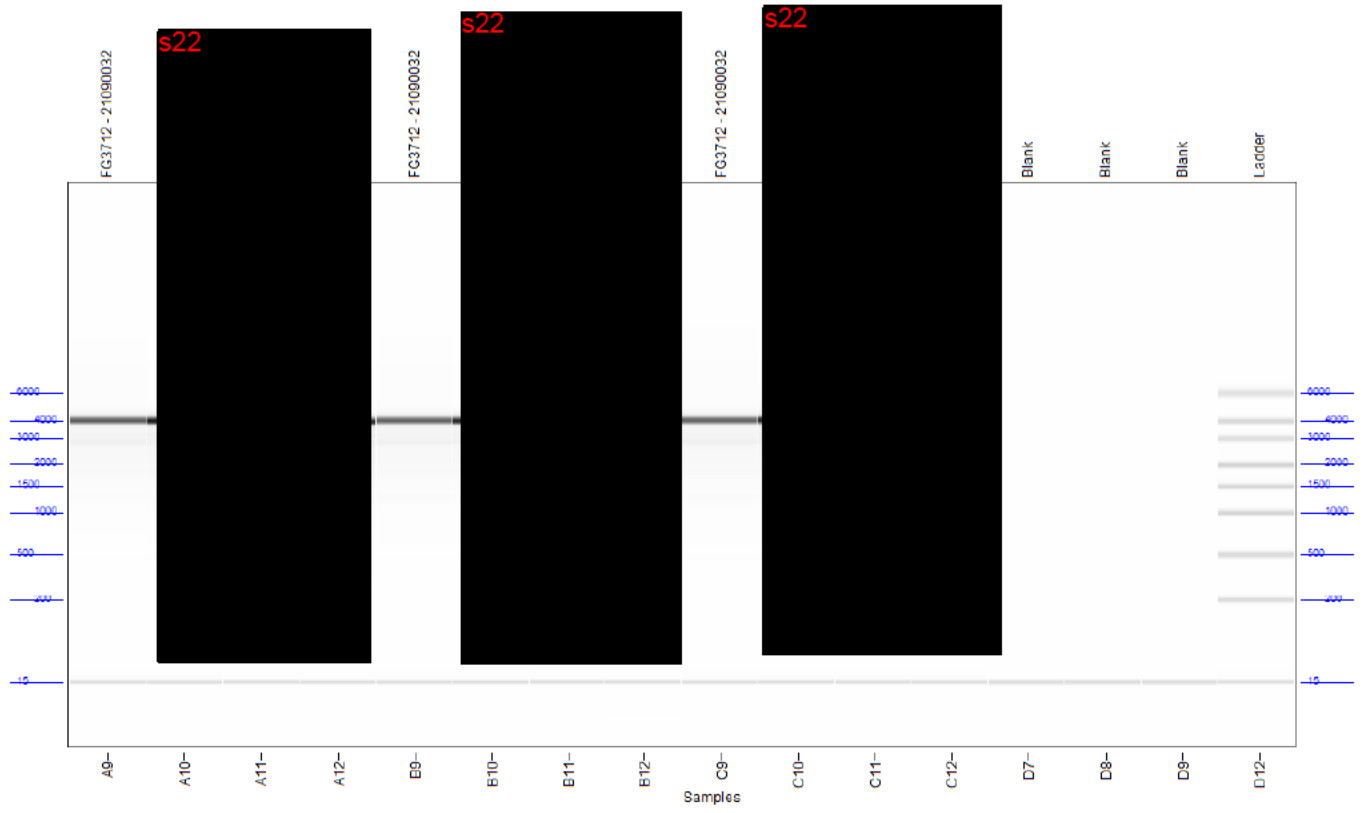
### Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds  
**Gel prime:** No  
**Full conditioning:** Yes  
**Gel prime to buffer:** Yes  
**Gel selection:** Gel 2  
**Perform prerun:** 8.0 kV, 30 sec.  
**Rinse:** No  
**Marker 1:** No  
**Rinse:** Tray: 3, Row: A, Dip count: 2  
**Sample injection:** 5.0 kV, 6 sec.  
**Separation:** 8.0 kV, 60.0 min.  
**Tray name:** Tray-1

**Analysis mode:** RNA (Eukaryotic)

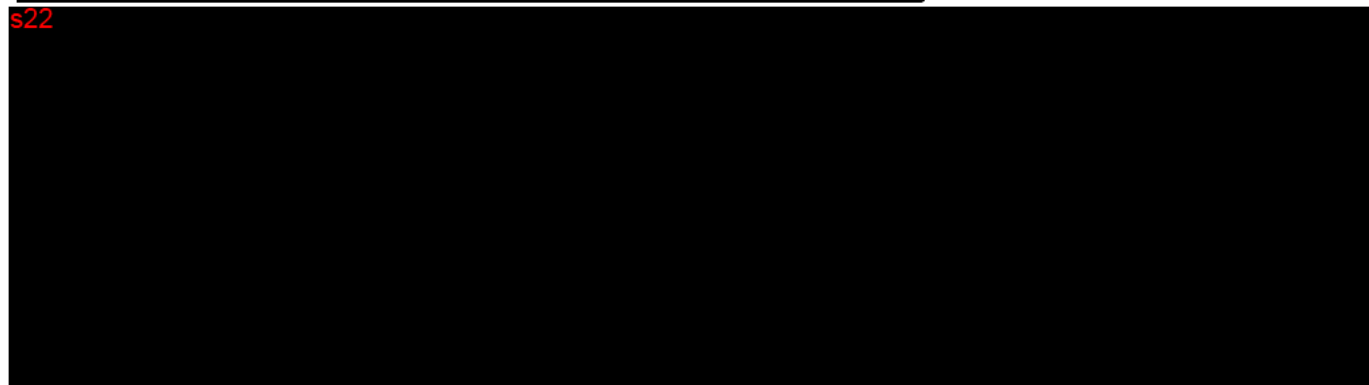
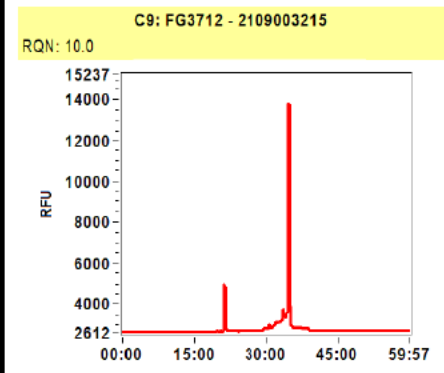
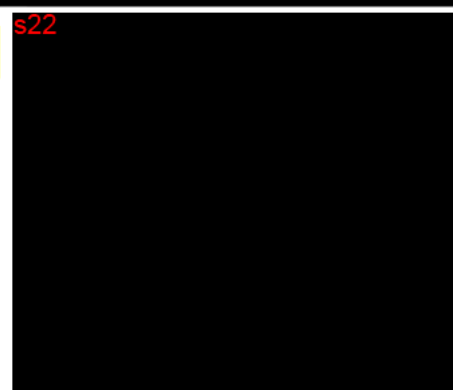
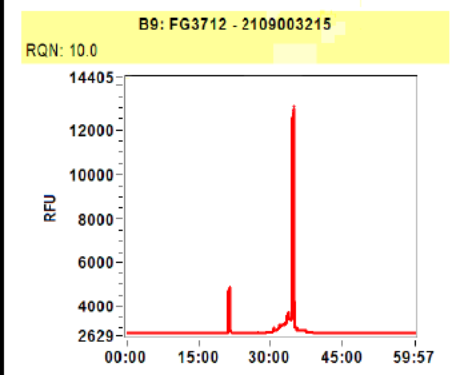
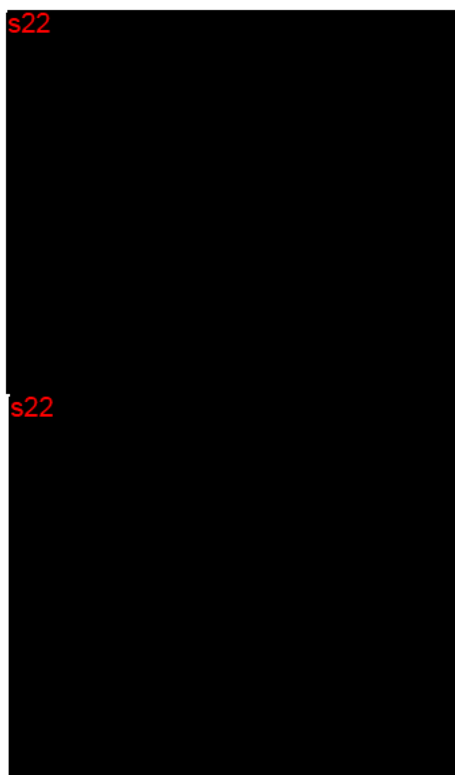
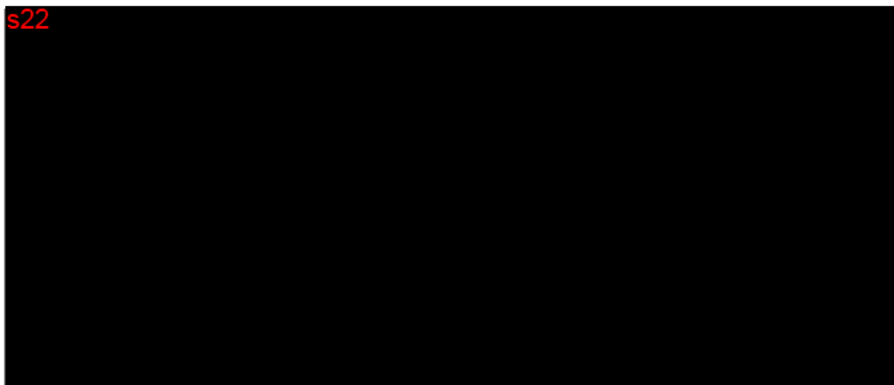
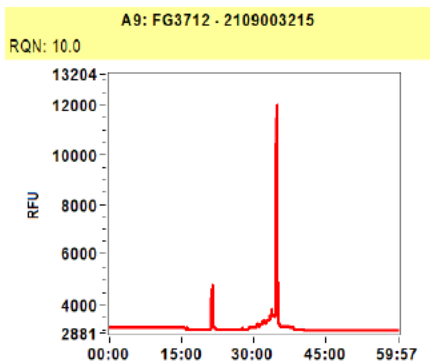
### Notes

### Gel Image

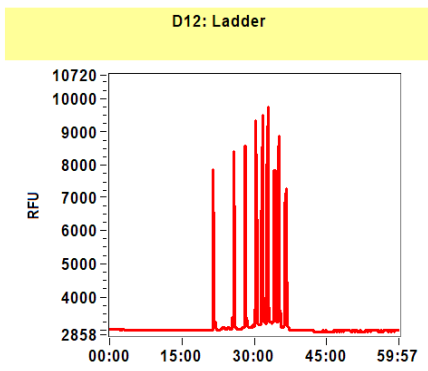
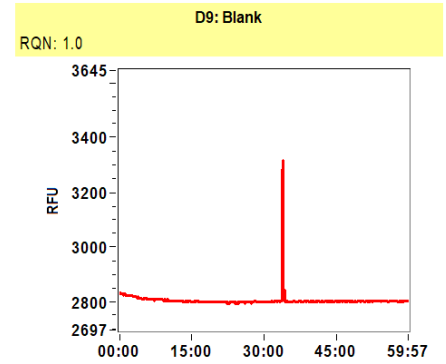
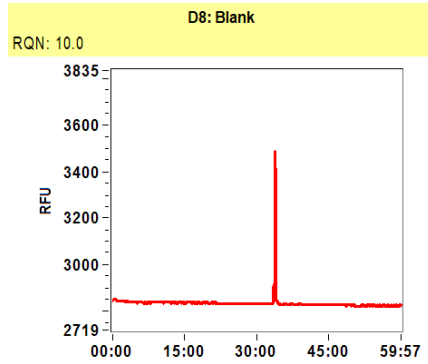
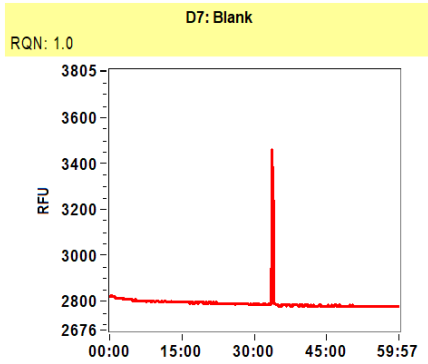




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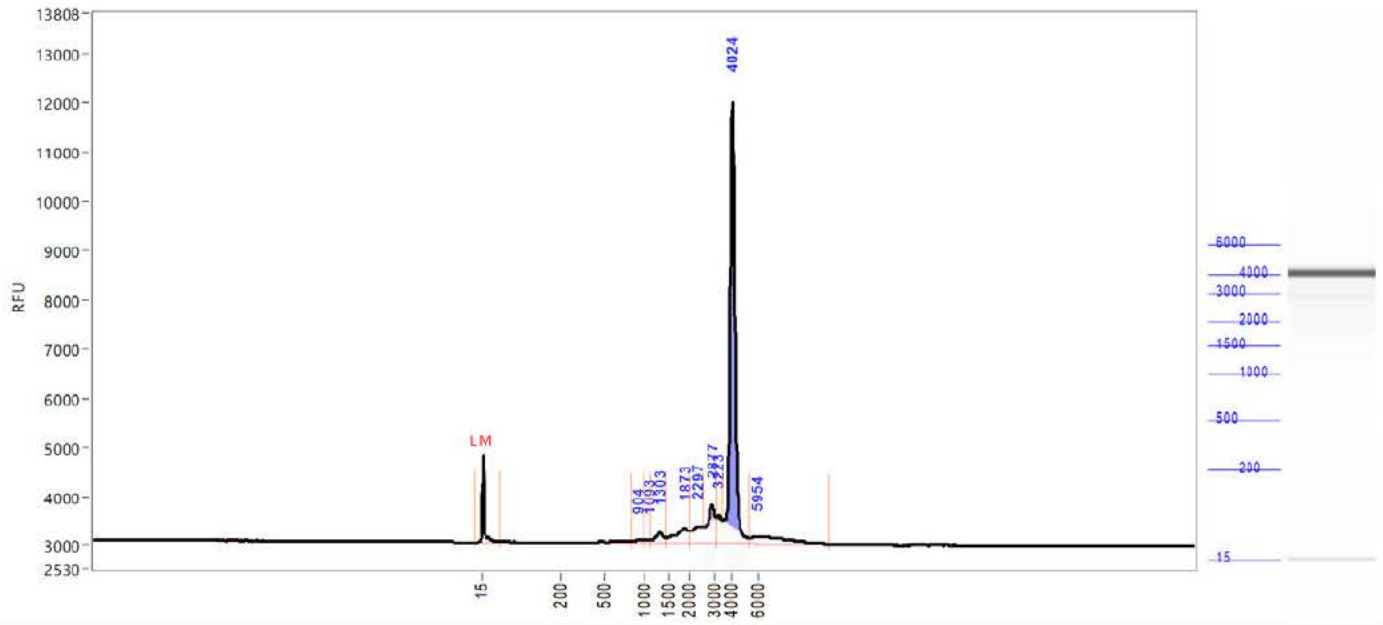


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Sample: EG3712 - 2109003215

s22



s47

s22



s22

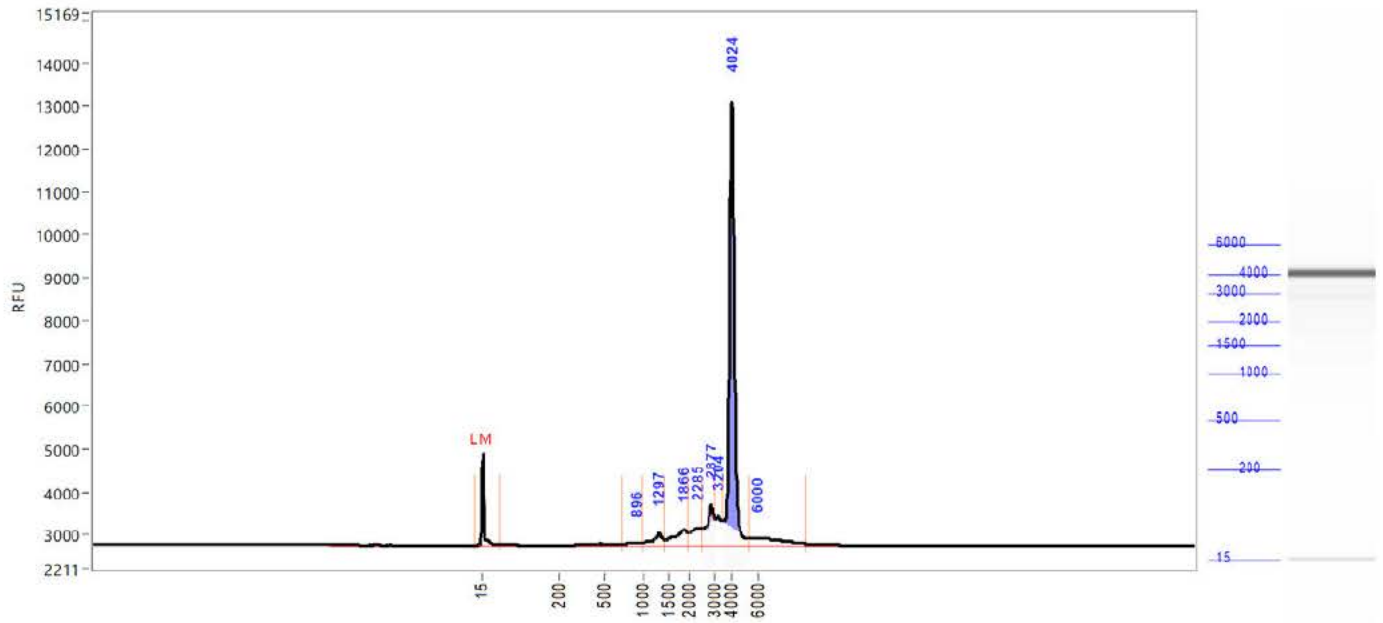


s22



Sample: FG3712 - 2109003215

s22



s47

s22





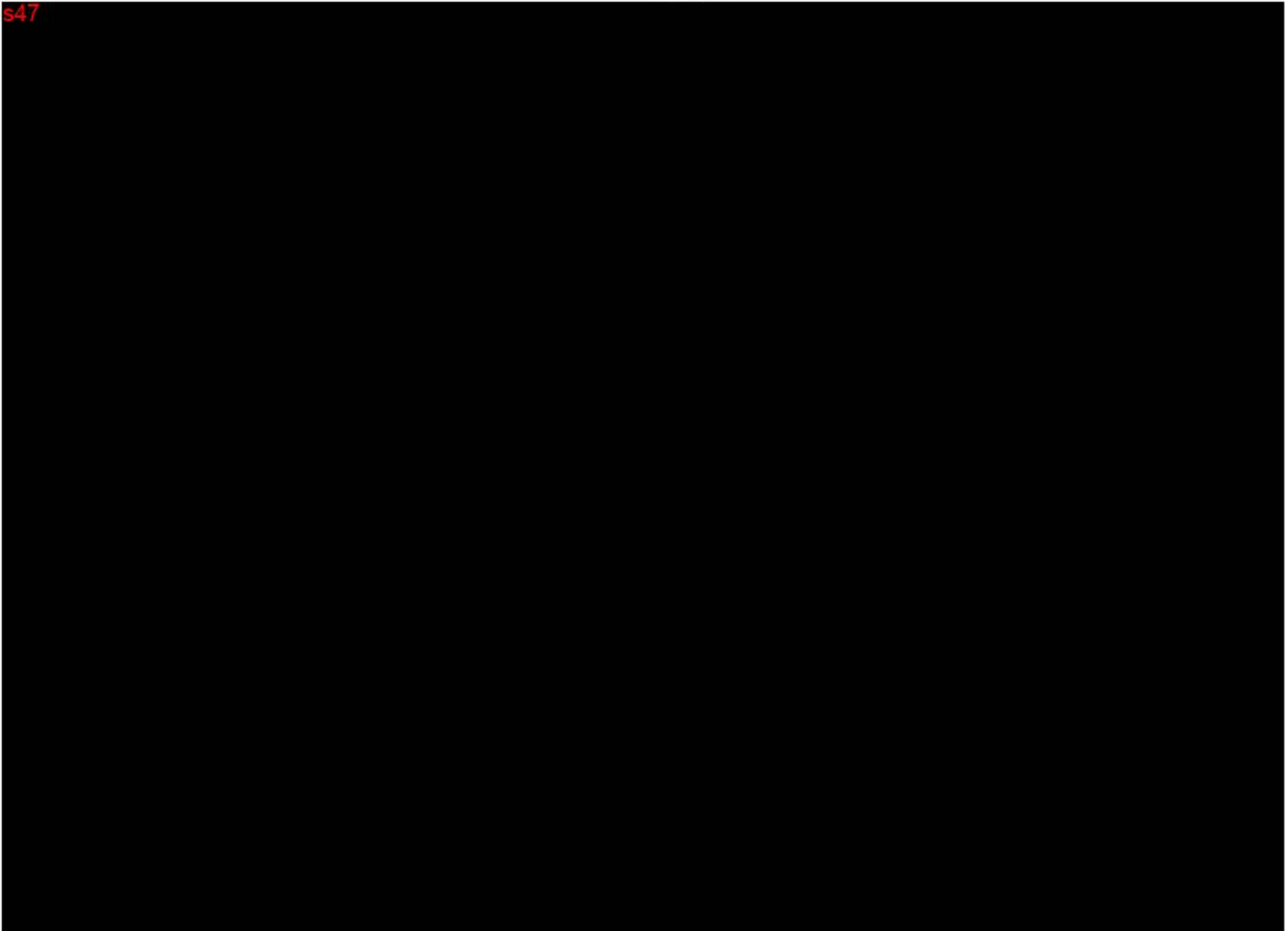
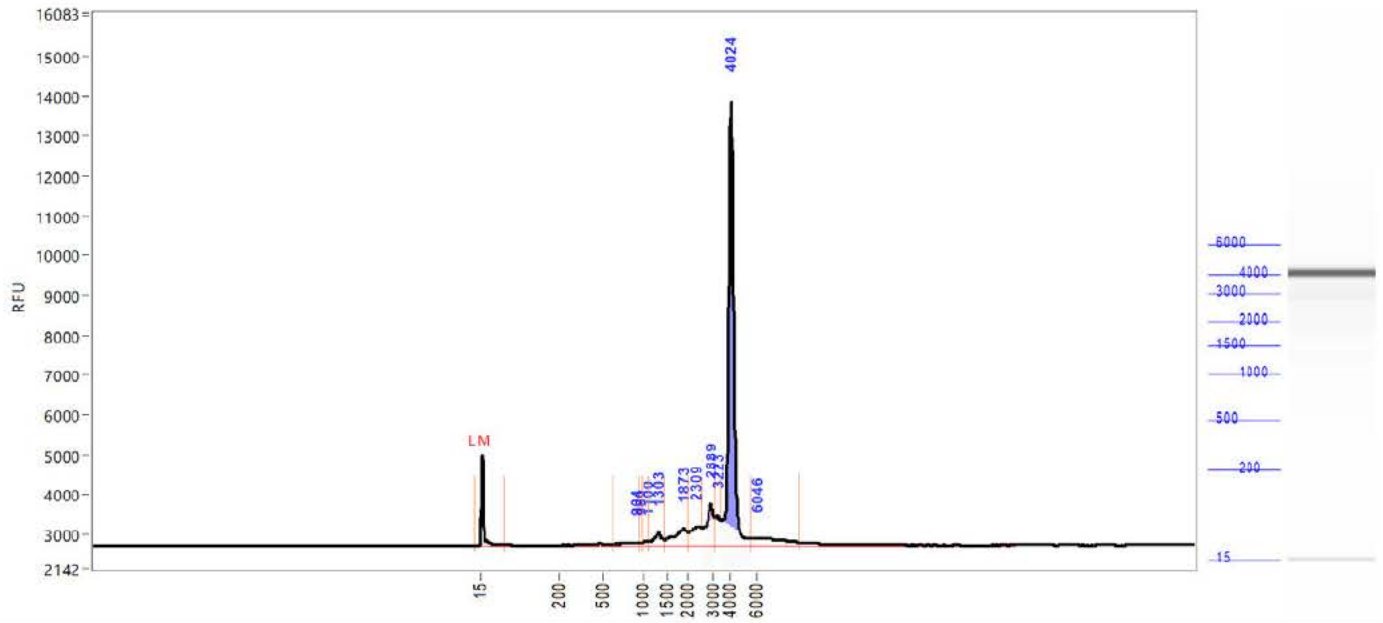
522



S22



Sample: FG3712 - 2109003215



S22



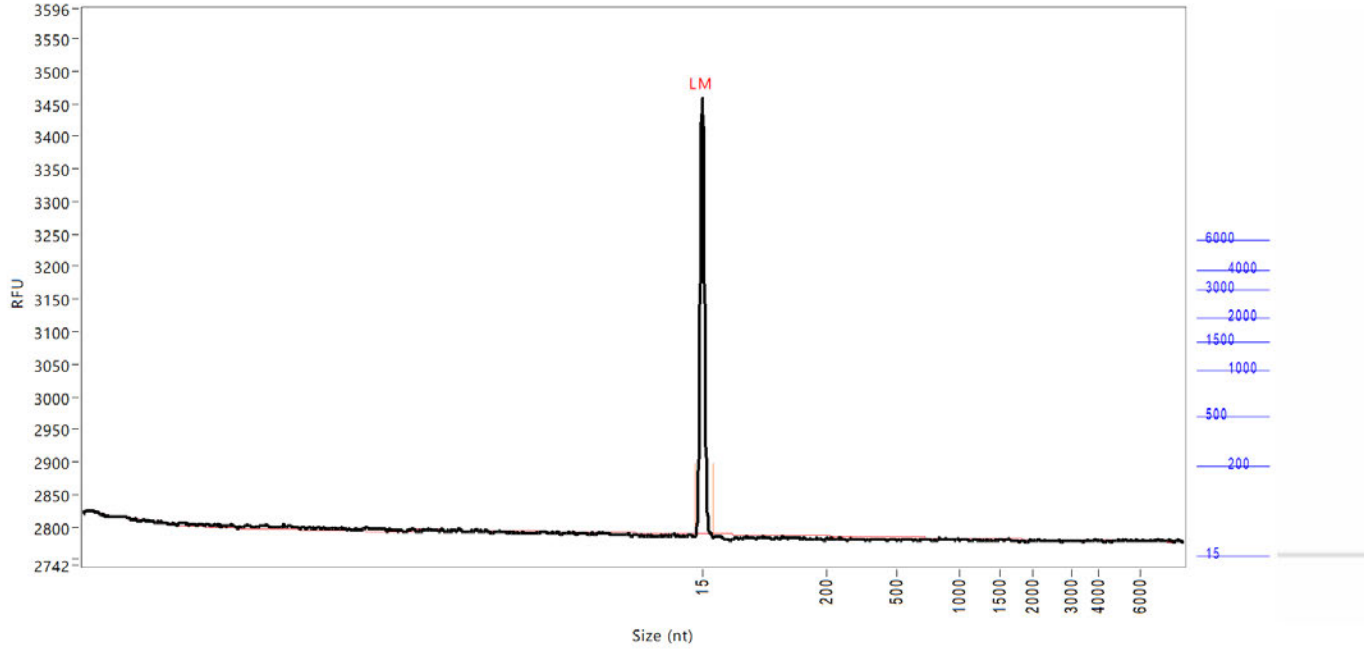
s22



S22



**Sample:** Blank  
**Well location:** D7  
**Created:** Tuesday, September 14, 2021 11:22:19 AM

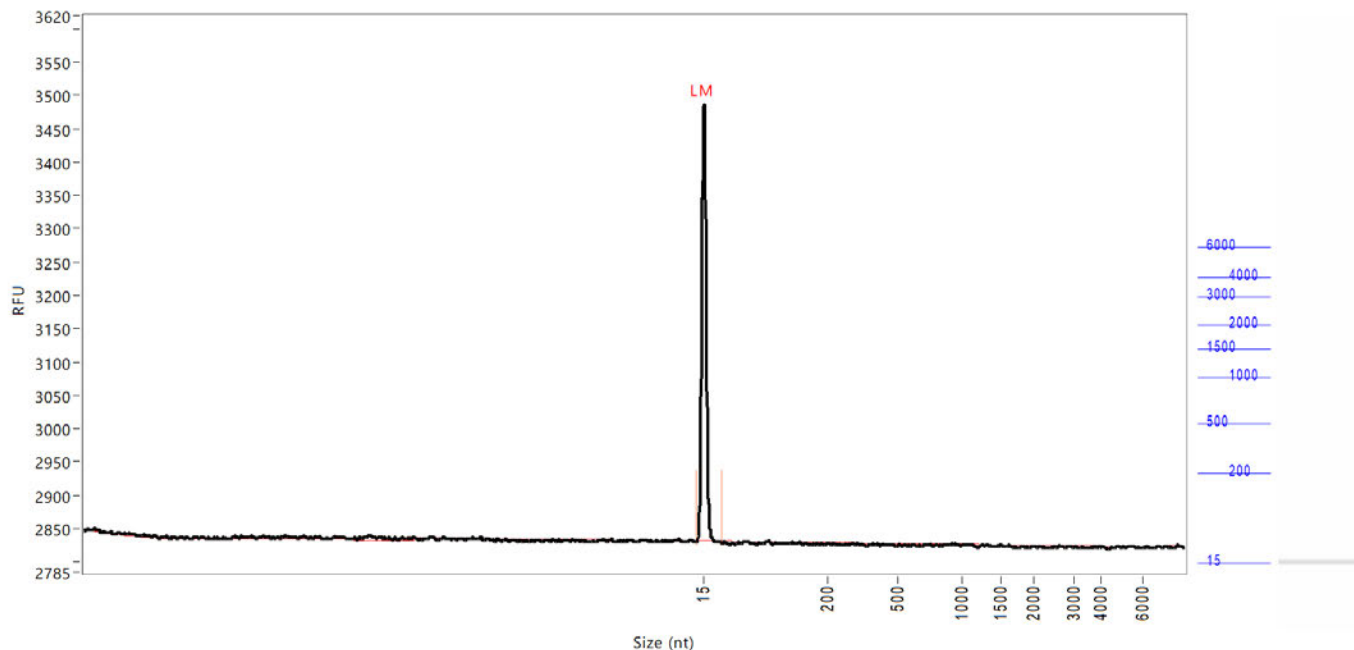


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.7825	4	32	668
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0031	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0031 ng/ul	100.0 %Total	0.0013 nmole/L	7176 Avg. Size (nt)	3.63 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Blank  
**Well location:** D8  
**Created:** Tuesday, September 14, 2021 11:22:19 AM



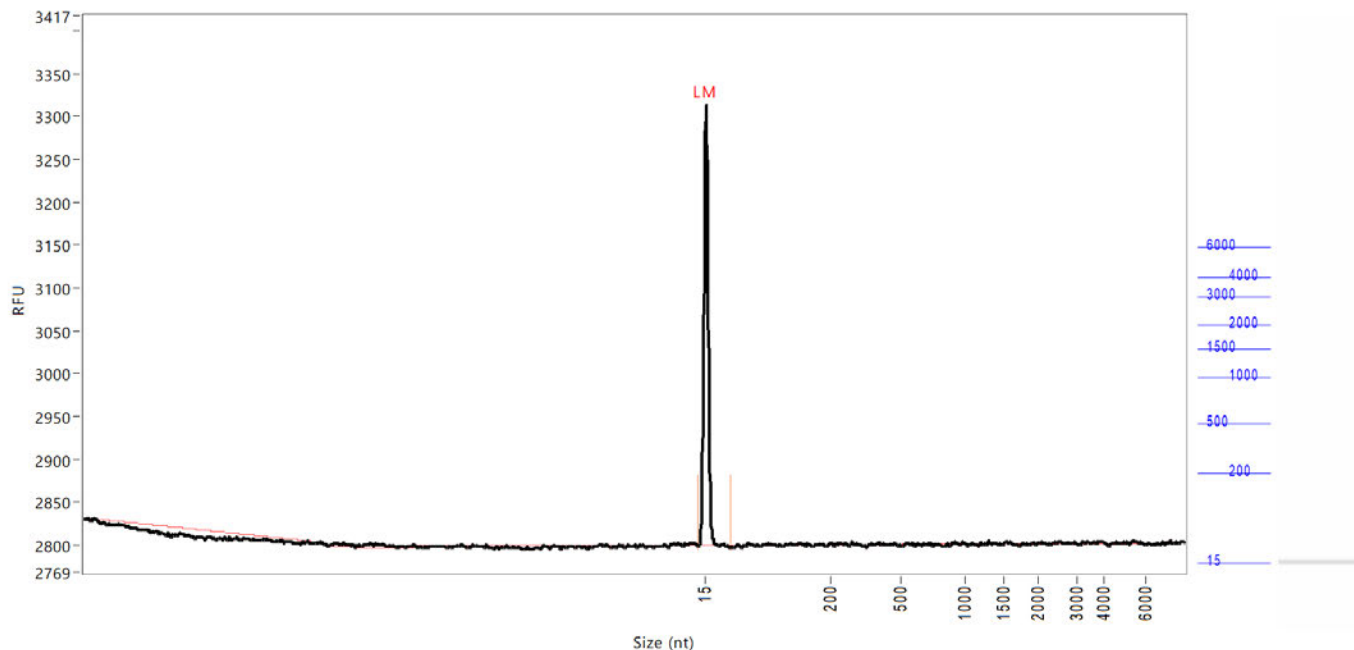
Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.7825	4	42	652
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0000	ng/uL		
	28s/18s:	0.0			
	RQN	10.0			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	NaN %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0000 ng/ul	NaN %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2



**Sample:** Blank  
**Well location:** D9  
**Created:** Tuesday, September 14, 2021 11:22:19 AM

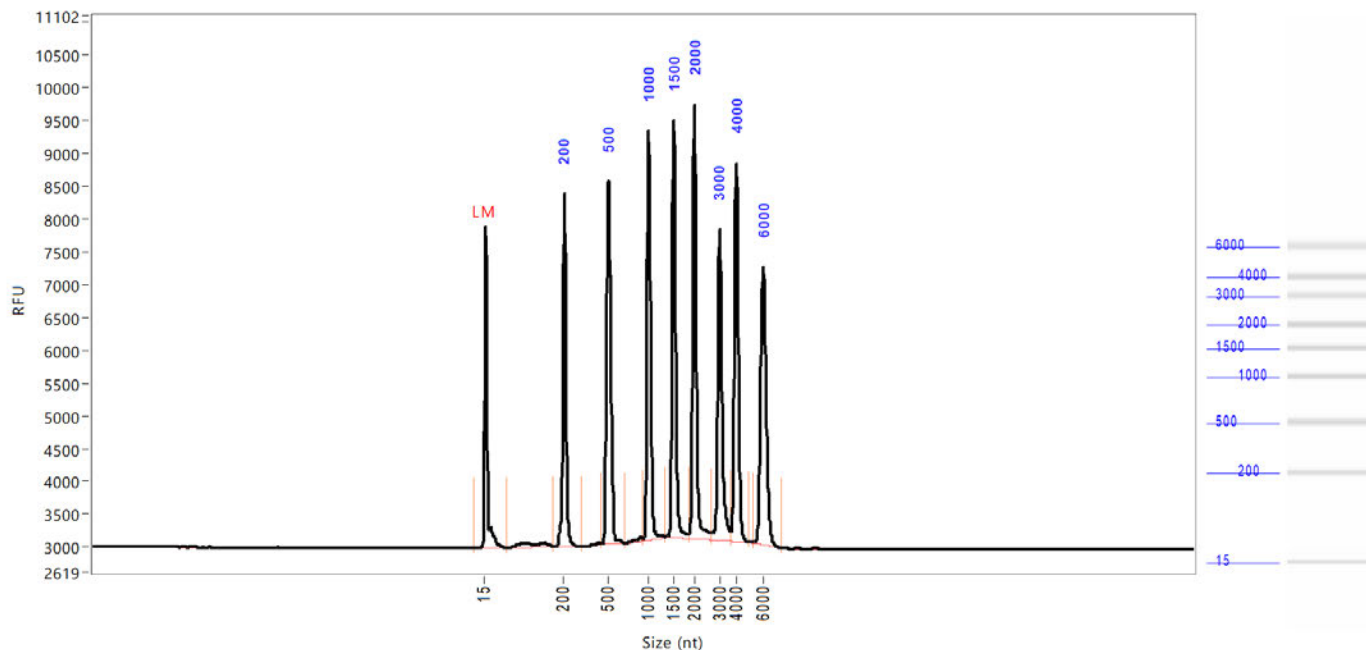


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.7825	4	52	511
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0195	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/uL	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0066 ng/uL	33.7 %Total	0.0038 nmole/L	5389 Avg. Size (nt)	1.29 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Tuesday, September 14, 2021 11:22:19 AM



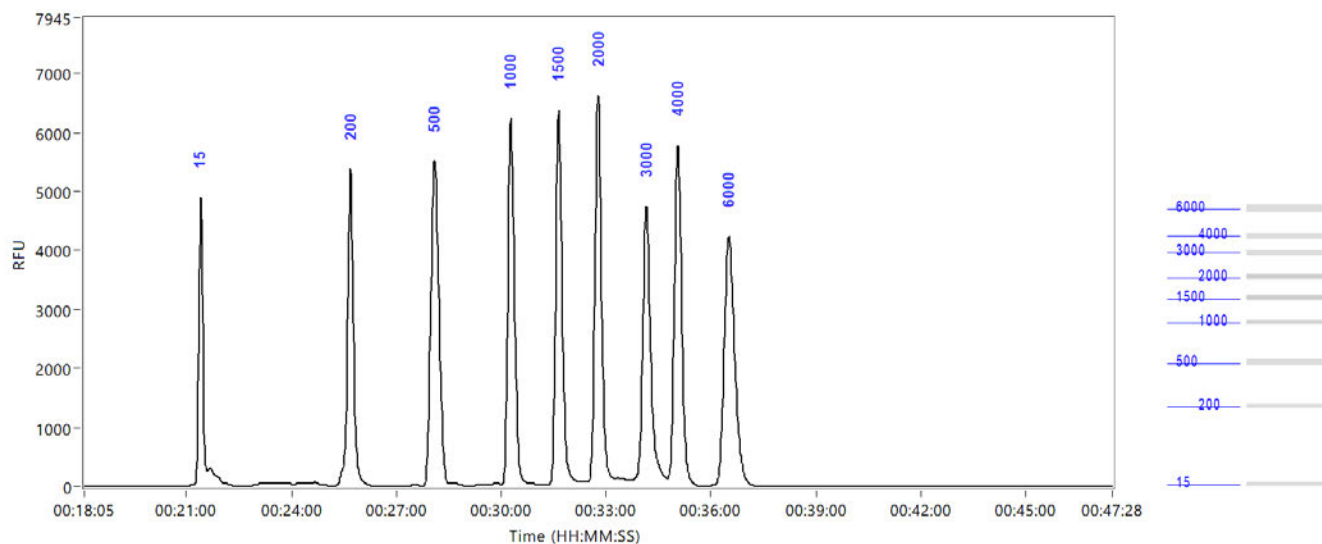
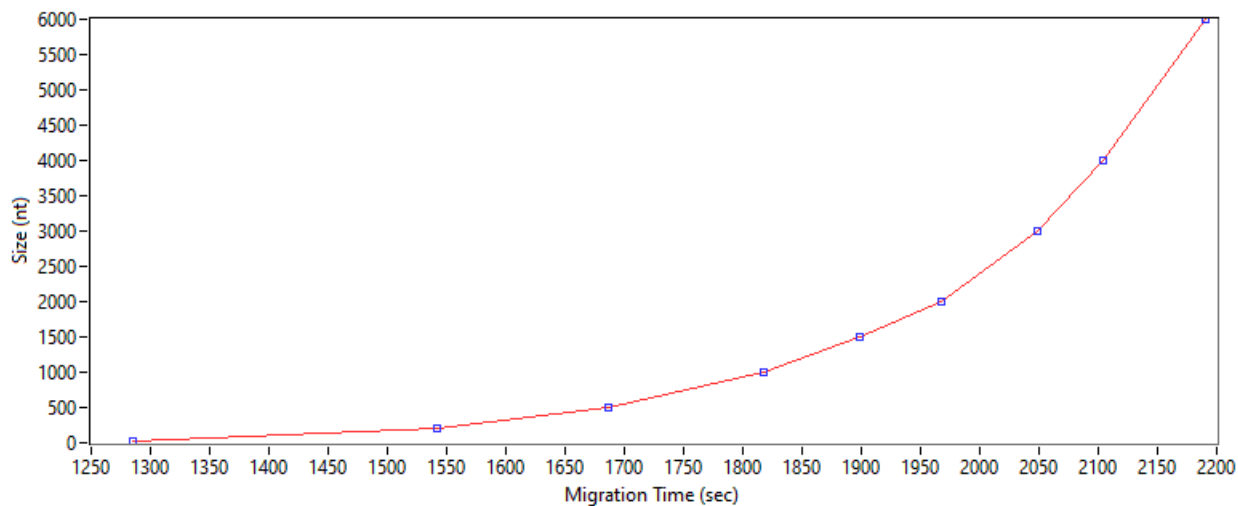
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.7825	0	66	4898
2	200	11.4822	174	320	5384
3	500	14.7817	452	712	5515
4	1000	12.1746	935	1321	6238
5	1500	11.8302	1321	1858	6363
6	2000	12.8585	1858	2679	6619
7	3000	10.4410	2679	3648	4739
8	4000	11.0765	3648	4884	5772
9	6000	11.1411	5303	7357	4244

TIC: 95.7857 ng/uL  
 TIM: 375.3307 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6      Sample min peak height: 200      Sample baseline V to V?: Y      Sample baseline V to V points: 3  
 Sample filter: Binomial      Number of points for filter: 9      Sample start region (min): 0      Sample end region (min): 60  
 Marker peak width (sec): 6      Marker min peak height: 100      Marker baseline V to V?: Y      Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU      Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder      Final concentration (ng/uL): 8.0000      Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Tuesday, September 14, 2021 11:22:19 AM  
**Fit type:** Point to point

Calibration curve





Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Appendix 1 - Fragment Analyzer Worksheet - Pfizer COMIRNATY	

### Worksheet for Fragment Analyzer - RNA Integrity

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-26	<b>Analysist</b>	s22
<b>TRIM link to data files</b>	D21-3088784, D21-3088783	<b>Test Date</b>	14/09/2021

Pipettes & Equipment	
Name	LIMS#
30-300 µL 12 channel pipette	N/A
p10 pipette	32835
p50 pipette	N/A
p100 pipette	32792
p200 pipette	N/A
Thermomixer	23660
Thermocycler	23865
P20	32891

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	N/A
Standard Sensitivity (SS) RNA kit Part 1 stored at 2-8°C	DNF-471-0500	6599311	2/02/2022
<i>Extra Blank solution</i>	<i>DNF-300-0008</i>	6595951	10/03/2022
Standard Sensitivity (SS) RNA kit Part 2 stored at -20°C (Diluent Marker & Intercalating dye)	Enter text.	Enter text.	Enter a date.
<i>Extra Diluent marker</i>	<i>DNF-369-0004</i>	6602442	7/04/2023
Standard Sensitivity (SS) RNA kit Part 3 stored at -70°C (RNA Ladder)	DNF-382-U020	0006600148	29/03/2022
Capillary conditioning solution	DNF-475-0100	6598614	22/03/2022
DEPC water	AM9961	2004017	N/A
20% Triton-X100 / 30% Ethanol solution	In house	MC1SEP21-01	1/02/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Intercalating dye	Dnf-600-u030	6603014	9/04/2022
Enter text.	Enter text.	Enter text.	Enter a date.

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT (Drawer B)	14/09/2021	15/09/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT (Drawer M)	14/09/2021	15/09/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT (Drawer 3)	31/08/2021	14/09/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	14/09/2021	28/09/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	14/09/2021	16/09/2021  48 hours
<b>Empty waste tray and waste bottle</b> <b>Reagents can be scaled up if required – this table provides the minimum for a single run.</b> <b>Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</b> <b>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</b>  <b>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</b>			

#### 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BF-25	BF-25	BF-25	BF-25	BF-25	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	BF-25	BF-25	BF-25	BF-25	BF-25	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	BF-25	BF-25	BF-25	BF-25	BF-25	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	L

**S1-6** = Samples in triplicate (a, b or c),

**RM** = Reference material

**BF-25** = Blank solution provided in kit,

**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test. Any samples not included in the test must be crossed off the plate layout, and results table below

System Suitability Criteria – RNA Ladder			
Plate location (wells)	D12		
Parameter	Limits	Results	Comments
RNA ladder profile	Visually comparable to figure 4 of SOP	ok	PASS
All peaks present	15 200 500 1000 1500 2000 3000 4000 6000 nt	ok	PASS
Peak heights	<60000 RFU	ok	PASS
Assay Acceptance Criteria – Reference Material			
Plate location (wells)	A12 B12 C12		
LIMS #	2108002914		
BATCH #	EE8493		
EXPIRY	5/02/2022		
Parameter	Limits	Results	Comments
Profile	Visually comparable to DP electropherogram in SOP	Ok/ok/ok	PASS
Migration time	Approximately comparable to profile in SOP	3963/4140/4047	PASS
Lower marker present	LM peak	Ok/ok/ok	PASS
Peak heights	5000-600000 for 2/3 replicates	13638/11564/11078	PASS
No negative peaks or baseline shifts	No significant peaks/shifts	Ok/ok/ok	PASS
Reference Material Dilutions / Calculation / Notes			
thaw date: 13/09/21 270ng/uL = 20 uL of 530 ng/uL master stock + 19 uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution			

Sample 1 Details	
Plate location (wells)	A11 B11 C11
LIMS #	2109003288
BATCH #	s22
EXPIRY	31/12/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4024/4047/4094	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	13736/14784/14515	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
Thaw date – 13/09/2021 – stored cell culture fridge , opened for the first time 13/09/21, stored at 2-8C 270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 2 Details	
Plate location (wells)	A10 B10 C10
LIMS #	2109003252
BATCH #	s22
EXPIRY	31/12/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4001/4001/4047	PASS
Lower marker	LM must be present	Ok/okok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	13857/14031/12549	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
Thaw date – 13/09/2021 – stored cell culture fridge , opened for the first time 13/09/21, stored at 2-8C 270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22



Sample 3 Details	
Plate location (wells)	A9 B9 C9
LIMS #	2109003215
BATCH #	FG3712
EXPIRY	30/11/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4024/4024/4024	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	8969/10350/11564	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
Thaw date – 13/09/2021 – stored cell culture fridge , opened for the first time 13/09/21, stored at 2-8C 270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 4 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Enter text.	Enter text.	Choose an item.
Lower marker	Enter text.	Enter text.	Choose an item.
Peak heights	Enter text.	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

Sample 5 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Enter text.	Enter text.	Choose an item.
Lower marker	Enter text.	Enter text.	Choose an item.
Peak heights	Enter text.	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

Sample 6 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Enter text.	Enter text.	Choose an item.
Lower marker	Enter text.	Enter text.	Choose an item.
Peak heights	Enter text.	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

**Notes**

Enter text.



Owner: s22	Number: Bio-BEE-Form-42
Author: s22	Version: 1
Active: 2/07/2021	Review: <QPulse_DocReviewDate>
Title: Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 13Sep2021

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899LRW Expiry: 18 March 2022Other Reagent: Pyrospere Batch# 0000904583  
September 2021Expiry: 29 June 2022 Use By: 20

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
Pfizer - Comirnaty covid vaccine	<u>FG3712</u>	<u>30 November 2021</u>	<u>2109003215-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
=	s47	<u>2500</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of vaccine</u>	<u>975uL</u>	<u>5uL Pyrospere</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrospere</u>
<u>N/A</u>	=	=	=
<u>N/A</u>	=	=	=
<u>N/A</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>N/A (Undefined)</u>	s47	<u>1.04</u>	<u>98</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

### Notes:

Click or tap here to enter text.

Data from Smear Analysis Table

1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate
2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the two smear sets.
3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	A1	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	NaN	NaN
A1	A1	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
A2	A2	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN
A2	A2	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN
A3	A3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A3	A3	Blank	4800 nt to 13000 nt	0.0062	35.1	0.0021	9276	1.24
A4	A4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A4	A4	Blank	4800 nt to 13000 nt	0.013	4.5	0.0043	9391	2.59
A5	A5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A5	A5	Blank	4800 nt to 13000 nt	0	100	0	8749	0
A6	A6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A6	A6	Blank	4800 nt to 13000 nt	0.0087	1.5	0.003	9017	3.49
A7	A7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3824	1.65
A7	A7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
§22								
A10	A10	FG3716-2109003	§47					
A10	A10	FG3716-2109003						
§22								
B1	B1	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3921	3.99
B1	B1	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	8927	40.87
B2	B2	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3901	3.34
B2	B2	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
B3	B3	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3890	2.99
B3	B3	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
B4	B4	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3883	3.01
B4	B4	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
B5	B5	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3876	2.91
B5	B5	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
B6	B6	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3850	1.95
B6	B6	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
B7	B7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	4182	7.92
B7	B7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	7725	22.22
§22								
B10	B10	FG3716-2109003	§47					
B10	B10	FG3716-2109003						
§22								
C1	C1	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3865	3.38
C1	C1	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
C2	C2	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3866	3.12
C2	C2	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	8956	34.26
C3	C3	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3852	2.88
C3	C3	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
C4	C4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C4	C4	Blank	4800 nt to 13000 nt	0.0229	3.9	0.0079	9039	0.33
C5	C5	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3833	2.21
C5	C5	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	11237	0.1
C6	C6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C6	C6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C7	C7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3806	1.85
C7	C7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
§22								
C10	C10	FG3716-2109003	§47					
C10	C10	FG3716-2109003						
§22								
D1	D1	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3838	2.42
D1	D1	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
D2	D2	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3833	2.29
D2	D2	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN
D3	D3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	4800 nt to 13000 nt	0.0054	28.1	0.0018	9064	0.23
D4	D4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	4800 nt to 13000 nt	0.0032	12	0.0011	8885	2.43
D5	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	4800 nt to 13000 nt	0.0012	7.1	0.0004	9043	0.18
D6	D6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D6	D6	Blank	4800 nt to 13000 nt	0.0445	75	0.0174	7991	13.48
D7	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	4800 nt to 13000 nt	0.0098	100	0.0032	9408	1.52
D8	D8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	4800 nt to 13000 nt	0.0031	29.3	0.0011	9236	3.72
D9	D9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D9	D9	Blank	4800 nt to 13000 nt	0.0092	100	0.0032	8945	3.11
D10	D10	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D10	D10	Blank	4800 nt to 13000 nt	0.0804	34.9	0.0312	8039	4.78
D11	D11	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D11	D11	Blank	4800 nt to 13000 nt	0.0038	16.1	0.0015	8178	5.46
D12	D12	Ladder						



Written By s22  
Authorised s22

Date Validated 10/06/2021  
Validation Due 10/06/2022

Revision no. 1  
LIMS number 33325

Validation Status **Validation OVERDUE**  
Analyst s22  
Assay Date 27/09/2021

Pass/Fail Parameters

minimum	cut off	maximum
s47		
result >	s47	

										% INTEGRITY SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
1	A1	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	NaN	NaN	Blank	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2	B1	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3921	3.99						
3	C1	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3865	3.38						
1	A2	Blank	3700 nt to 4800 nt	0	NaN	NaN	NaN	NaN	Blank	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2	B2	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3901	3.34						
3	C2	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3866	3.12						
1	A3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	#DIV/0!	#DIV/0!	FAIL	
2	B3	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3890	2.99						
3	C3	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3852	2.88						
1	A4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B4	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3883	3.01						
3	C4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	#DIV/0!	#DIV/0!	FAIL	
2	B5	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3876	2.91						
3	C5	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3833	2.21						
1	A6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B6	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3850	1.95						
3	C6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3824	1.65	Blank	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2	B7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	4182	7.92						
3	C7	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3806	1.85						
s22													s22	
1	A10	FG3716-2109003217							FG3716-2109003217				PASS	
2	B10	FG3716-2109003217												
3	C10	FG3716-2109003217												
s22														
1	D1	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3838	2.42	Blank	0.00	#DIV/0!	#DIV/0!	FAIL	
2	D2	Blank	3700 nt to 4800 nt	NaN	NaN	NaN	3833	2.29						
3	D3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	D4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	D6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	D8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	D9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						

										% LATE MIGRATING SPECIES SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
1	A1	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN	Blank	#DIV/0!	#DIV/0!	#DIV/0!		
2	B1	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	8927	40.87						
3	C1	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN						
1	A2	Blank	4800 nt to 13000 nt	0	NaN	NaN	NaN	NaN	Blank	#DIV/0!	#DIV/0!	#DIV/0!		
2	B2	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN						
3	C2	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	8956	34.26						
1	A3	Blank	4800 nt to 13000 nt	0.0062	35.1	0.0021	9276	1.24	Blank	35.10	#DIV/0!	#DIV/0!		
2	B3	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN						
3	C3	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN						
1	A4	Blank	4800 nt to 13000 nt	0.013	4.5	0.0043	9391	2.59	Blank	4.20	0.42	10.10		
2	B4	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN						
3	C4	Blank	4800 nt to 13000 nt	0.0229	3.9	0.0079	9039	0.33						
1	A5	Blank	4800 nt to 13000 nt	0	100	0	8749	0	Blank	100.00	#DIV/0!	#DIV/0!		
2	B5	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN						
3	C5	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	11237	0.1						
1	A6	Blank	4800 nt to 13000 nt	0.0087	1.5	0.003	9017	3.49	Blank	0.75	1.06	141.42		
2	B6	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN						
3	C6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN	Blank	#DIV/0!	#DIV/0!	#DIV/0!		
2	B7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	7725	22.22						
3	C7	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN						
s22														

822													
1	A10	FG3716-2109003217	847										
2	B10	FG3716-2109003217	FG3716-2109003217 847										
3	C10	FG3716-2109003217											
822													
1	D1	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN	Blank	28.10	#DIV/0!	#DIV/0!	
2	D2	Blank	4800 nt to 13000 nt	NaN	NaN	NaN	NaN	NaN					
3	D3	Blank	4800 nt to 13000 nt	0.0054	28.1	0.0018	9064	0.23					
1	D4	Blank	4800 nt to 13000 nt	0.0032	12	0.0011	8885	2.43					
2	D5	Blank	4800 nt to 13000 nt	0.0012	7.1	0.0004	9043	0.18	Blank	31.37	37.87	120.72	
3	D6	Blank	4800 nt to 13000 nt	0.0445	75	0.0174	7991	13.48					
1	D7	Blank	4800 nt to 13000 nt	0.0098	100	0.0032	9408	1.52					
2	D8	Blank	4800 nt to 13000 nt	0.0031	29.3	0.0011	9236	3.72	Blank	76.43	40.82	53.40	
3	D9	Blank	4800 nt to 13000 nt	0.0092	100	0.0032	8945	3.11					



VALIDATION DATA

Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2
A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4
A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4
A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6
A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8
A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8
A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10
A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1
A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12
A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2
A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14
A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
A8	sample8-rep1	3500 nt to 5389 nt	40	80	82.5	4079	16
A8	sample8-rep1	5389 nt to 13000 nt	4	8	10.5	6774	1.6
A9	sample9-rep1	3500 nt to 5389 nt	45	90	92.5	4053	18
A9	sample9-rep1	5389 nt to 13000 nt	4.5	9	11.5	6916	1.8
A10	sample10-rep1	3500 nt to 5389 nt	50	100	102.5	4045	20
A10	sample10-rep1	5389 nt to 13000 nt	5	10	12.5	6870	2
A11	sample11-rep1	3500 nt to 5389 nt	55	110	112.5	4089	22
A11	sample11-rep1	5389 nt to 13000 nt	5.5	11	13.5	7320	2.2
A12	sample12-rep1	3500 nt to 5389 nt	60	120	122.5	4061	24
A12	sample12-rep1	5389 nt to 13000 nt	6	12	14.5	7135	2.4
B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62
B8	sample8-rep2	3500 nt to 5389 nt	40.5	81	83.5	4091	16.2
B8	sample8-rep2	5389 nt to 13000 nt	4.55	9	11.6	5534	1.82
B9	sample9-rep2	3500 nt to 5389 nt	45.5	91	93.5	4061	18.2
B9	sample9-rep2	5389 nt to 13000 nt	5.05	10	12.6	4079	2.02
B10	sample10-rep2	3500 nt to 5389 nt	50.5	101	103.5	4033	20.2
B10	sample10-rep2	5389 nt to 13000 nt	5.55	11	13.6	6807	2.22
B11	sample11-rep2	3500 nt to 5389 nt	55.5	111	113.5	4069	22.2
B11	sample11-rep2	5389 nt to 13000 nt	6.05	12	14.6	7000	2.42
B12	sample12-rep2	3500 nt to 5389 nt	60.5	121	123.5	4067	24.2
B12	sample12-rep2	5389 nt to 13000 nt	6.55	13	15.6	7094	2.62
C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4
C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4
C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4
C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4
C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4
C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4
C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4
C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84
C8	sample8-rep3	3500 nt to 5389 nt	41	82	84.5	4089	16.4
C8	sample8-rep3	5389 nt to 13000 nt	5.1	10	12.7	5684	2.04
C9	sample9-rep3	3500 nt to 5389 nt	46	92	94.5	4065	18.4
C9	sample9-rep3	5389 nt to 13000 nt	5.6	11	13.7	5530	2.24
C10	sample10-rep3	3500 nt to 5389 nt	51	102	104.5	4037	20.4
C10	sample10-rep3	5389 nt to 13000 nt	6.1	12	14.7	6551	2.44
C11	sample11-rep3	3500 nt to 5389 nt	56	112	114.5	4061	22.4
C11	sample11-rep3	5389 nt to 13000 nt	6.6	13	15.7	6970	2.64
C12	sample12-rep3	3500 nt to 5389 nt	61	122	124.5	4070	24.4
C12	sample12-rep3	5389 nt to 13000 nt	7.1	14	16.7	6740	2.84
D1	sample13-rep1	3500 nt to 5389 nt	65	130	15.5	4079	2.6
D1	sample13-rep1	5389 nt to 13000 nt	2.15	13	6.8	4079	0.86
D2	sample13-rep2	3500 nt to 5389 nt	65.5	131	25.5	3757	4.6
D2	sample13-rep2	5389 nt to 13000 nt	2.65	14	7.8	9444	1.06
D3	sample13-rep3	3500 nt to 5389 nt	66	132	35.5	4079	6.6
D3	sample13-rep3	5389 nt to 13000 nt	3.15	15	8.8	4079	1.26
D4	sample14-rep1	3500 nt to 5389 nt	70	140	45.5	4079	8.6
D4	sample14-rep1	5389 nt to 13000 nt	3.65	14	9.8	4079	1.46
D5	sample14-rep2	3500 nt to 5389 nt	70.5	141	55.5	5026	10.6
D5	sample14-rep2	5389 nt to 13000 nt	4.15	15	10.8	6983	1.66
D6	sample14-rep3	3500 nt to 5389 nt	71	142	65.5	5240	12.6
D6	sample14-rep3	5389 nt to 13000 nt	4.65	16	11.8	6440	1.86
D7	sample15-rep1	3500 nt to 5389 nt	75	150	75.5	5240	14.6
D7	sample15-rep1	5389 nt to 13000 nt	5.15	15	12.8	6440	2.06
D8	sample15-rep2	3500 nt to 5389 nt	75.5	151	85.5	4079	16.6
D8	sample15-rep2	5389 nt to 13000 nt	5.65	16	13.8	4079	2.26
D9	sample15-rep3	3500 nt to 5389 nt	76	152	95.5	3757	18.6
D9	sample15-rep3	5389 nt to 13000 nt	6.15	17	14.8	9444	2.46
D10	Blank-rep1	3500 nt to 5389 nt	80	160	105.5	4079	20.6
D10	Blank-rep1	5389 nt to 13000 nt	6.65	16	15.8	4079	2.66
D11	Blank2-rep1	3500 nt to 5389 nt	80.5	161	115.5	4079	22.6
D11	Blank2-rep1	5389 nt to 13000 nt	7.15	17	16.8	4079	2.86
D12	Ladder	3500 nt to 5389 nt	81	162	125.5	5026	24.6
D12	Ladder	5389 nt to 13000 nt	7.65	18	17.8	6983	3.06

Pass/Fail Parameters  
 minimum cut off maximum  
 547  
 result >> 547

RESULTS FOR VALIDATION DATA

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				
									Sample ID	Average	stdev	%CV	
	1 A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2	sample1-rep1	11.00	1.00	9.09	FAIL
	2 B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2					
	3 C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4					
	1 A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4	sample2-rep1	21.00	1.00	4.76	FAIL
	2 B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2					
	3 C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4					
	1 A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6	sample3-rep1	31.00	1.00	3.23	FAIL
	2 B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2					
	3 C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4					
	1 A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8	sample4-rep1	41.00	1.00	2.44	FAIL
	2 B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2					
	3 C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4					
	1 A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10	sample5-rep1	51.00	1.00	1.96	FAIL
	2 B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2					
	3 C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4					
	1 A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12	sample6-rep1	61.00	1.00	1.64	PASS
	2 B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2					
	3 C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4					
	1 A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14	sample7-rep1	71.00	1.00	1.41	PASS
	2 B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2					
	3 C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4					
	1 A8	sample8-rep1	3500 nt to 5389 nt	40	80	82.5	4079	16	sample8-rep1	81.00	1.00	1.23	PASS
	2 B8	sample8-rep2	3500 nt to 5389 nt	40.5	81	83.5	4091	16.2					
	3 C8	sample8-rep3	3500 nt to 5389 nt	41	82	84.5	4089	16.4					
	1 A9	sample9-rep1	3500 nt to 5389 nt	45	90	92.5	4053	18	sample9-rep1	91.00	1.00	1.10	PASS
	2 B9	sample9-rep2	3500 nt to 5389 nt	45.5	91	93.5	4061	18.2					
	3 C9	sample9-rep3	3500 nt to 5389 nt	46	92	94.5	4065	18.4					
	1 A10	sample10-rep1	3500 nt to 5389 nt	50	100	102.5	4045	20	sample10-rep1	101.00	1.00	0.99	PASS
	2 B10	sample10-rep2	3500 nt to 5389 nt	50.5	101	103.5	4033	20.2					
	3 C10	sample10-rep3	3500 nt to 5389 nt	51	102	104.5	4037	20.4					
	1 A11	sample11-rep1	3500 nt to 5389 nt	55	110	112.5	4089	22	sample11-rep1	111.00	1.00	0.90	PASS
	2 B11	sample11-rep2	3500 nt to 5389 nt	55.5	111	113.5	4069	22.2					
	3 C11	sample11-rep3	3500 nt to 5389 nt	56	112	114.5	4061	22.4					
	1 A12	sample12-rep1	3500 nt to 5389 nt	60	120	122.5	4061	24	sample12-rep1	121.00	1.00	0.83	PASS
	2 B12	sample12-rep2	3500 nt to 5389 nt	60.5	121	123.5	4067	24.2					
	3 C12	sample12-rep3	3500 nt to 5389 nt	61	122	124.5	4070	24.4					
	1 D1	sample13-rep1	3500 nt to 5389 nt	65	130	15.5	4079						

VALIDATION DATA								
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62
3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84

RESULTS FOR VALIDATION DATA										% INTEGRITY SUMMARY				
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV		Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2		sample1-rep1	11.0	1.0	9.1	FAIL
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2						
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4						
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4		sample2-rep1	21.0	1.0	4.8	FAIL
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2						
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4						
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6		sample3-rep1	31.0	1.0	3.2	FAIL
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2						
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4						
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8		sample4-rep1	41.0	1.0	2.4	FAIL
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2						
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4						
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10		sample5-rep1	51.0	1.0	2.0	FAIL
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2						
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4						
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12		sample6-rep1	61.0	1.0	1.6	PASS
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2						
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4						
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14		sample7-rep1	71.0	1.0	1.4	PASS
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2						
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4						

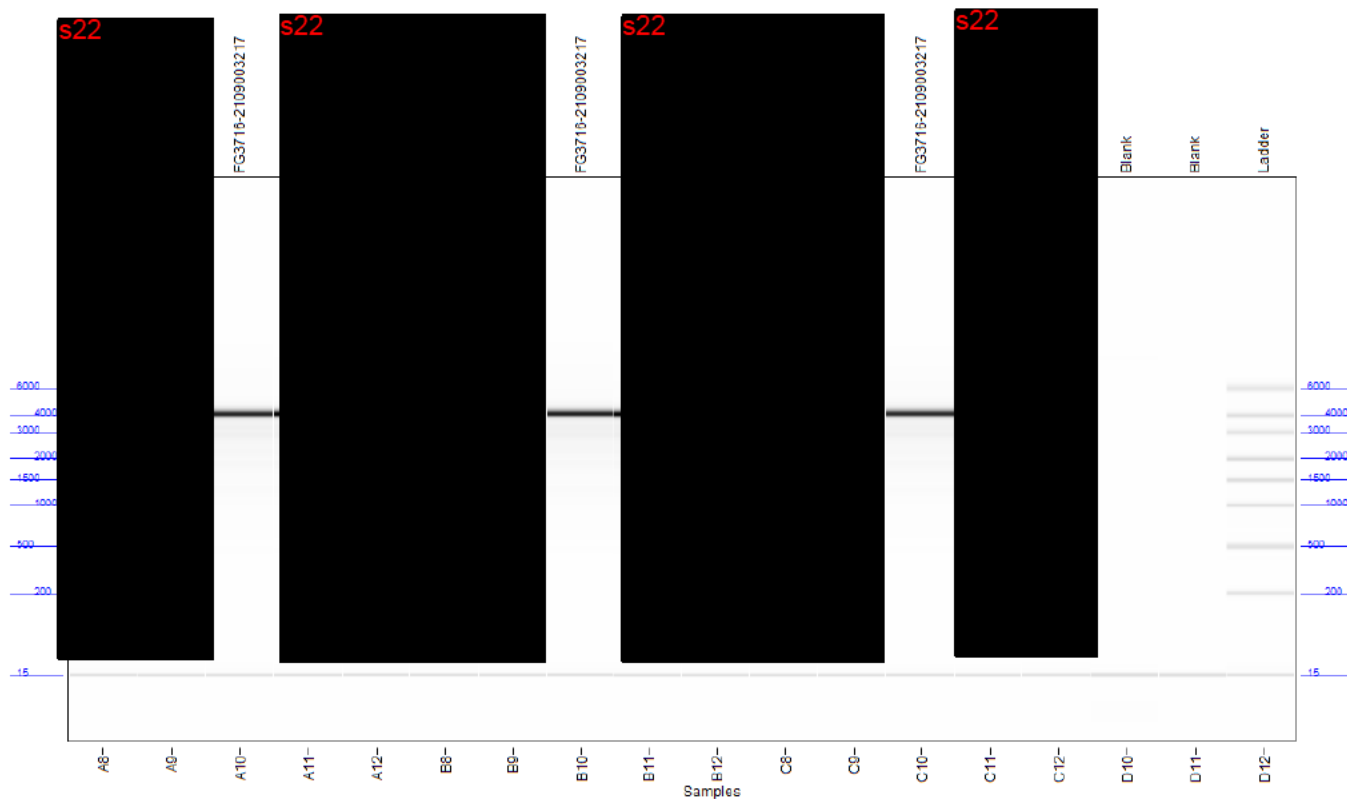
  

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV		Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2		sample1-rep1	2.0	1.0	50.0	
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42						
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64						
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4		sample2-rep1	3.0	1.0	33.3	
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62						
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84						
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6		sample3-rep1	4.0	1.0	25.0	
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82						
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04						
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8		sample4-rep1	5.0	1.0	20.0	
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02						
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24						
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1		sample5-rep1	6.0	1.0	16.7	
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22						
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44						
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2		sample6-rep1	7.0	1.0	14.3	
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42						
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64						
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4		sample7-rep1	8.0	1.0	12.5	
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62						
3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84						

## **Instrument controller software run summary:**

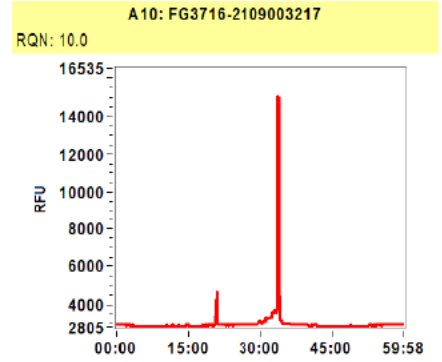
**Analysis mode:** RNA (Eukaryotic)

### Gel Image



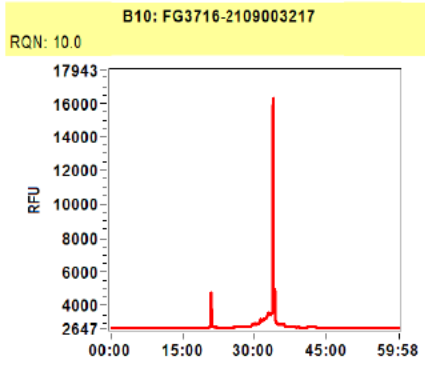
s22

s22



s22

s22

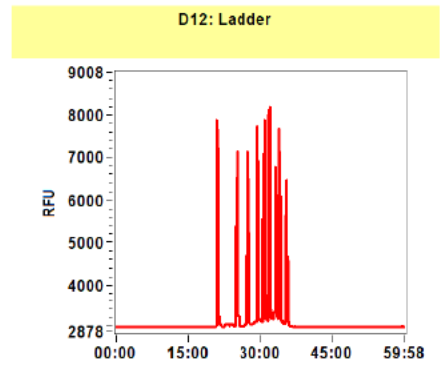
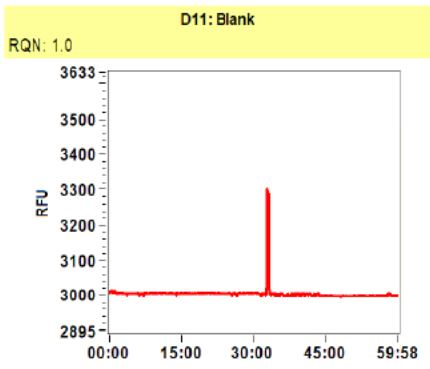
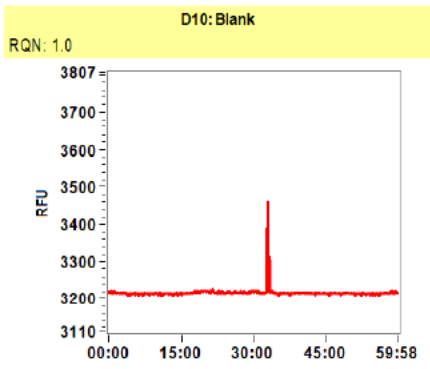
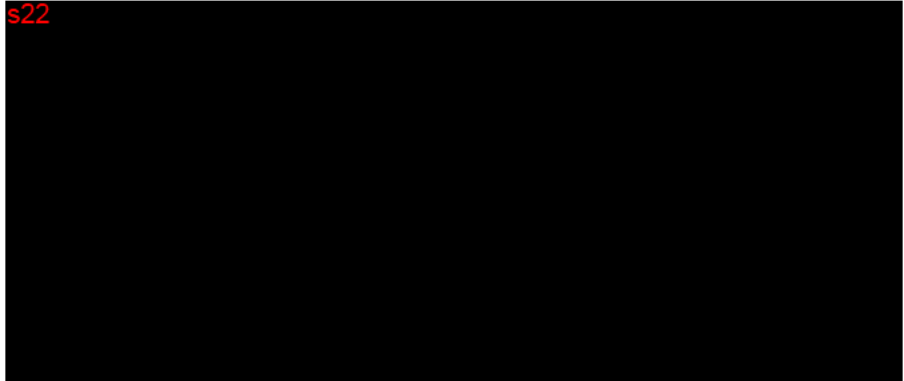
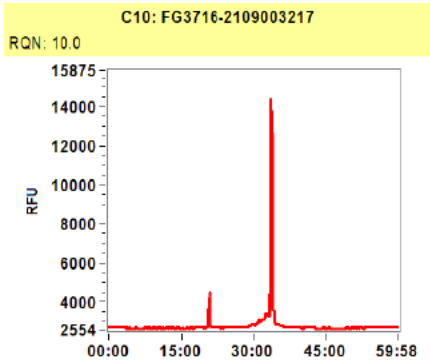


s22

s22



s22



S22



S22

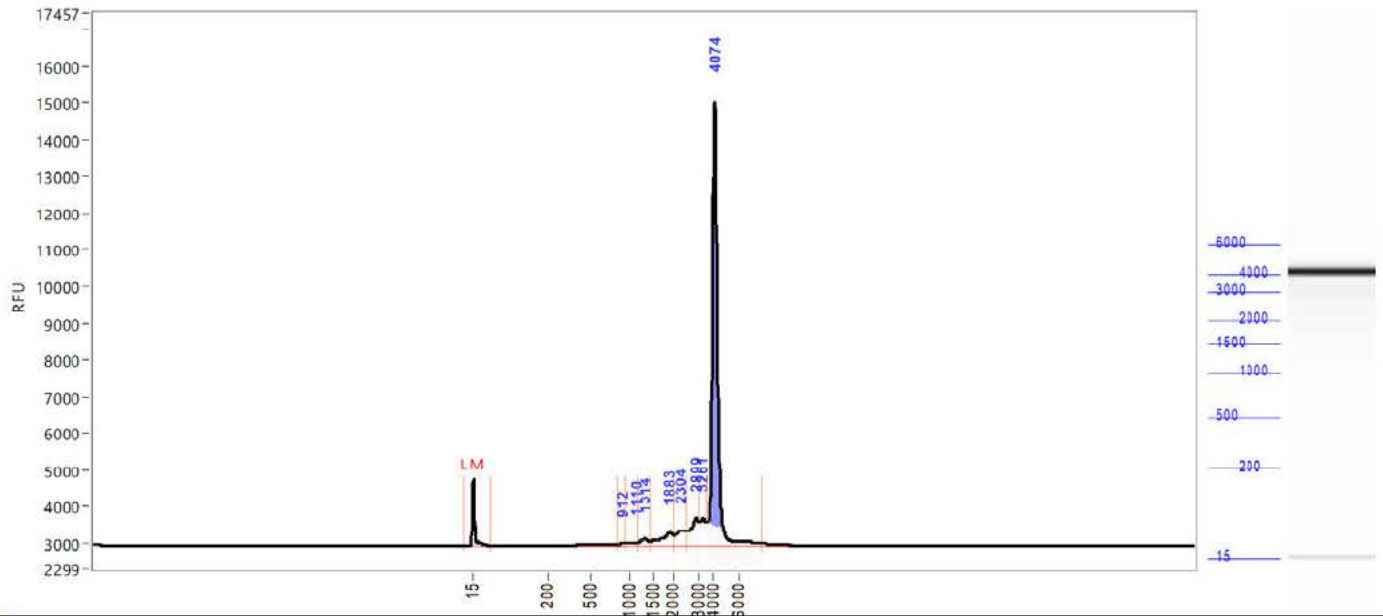


S22

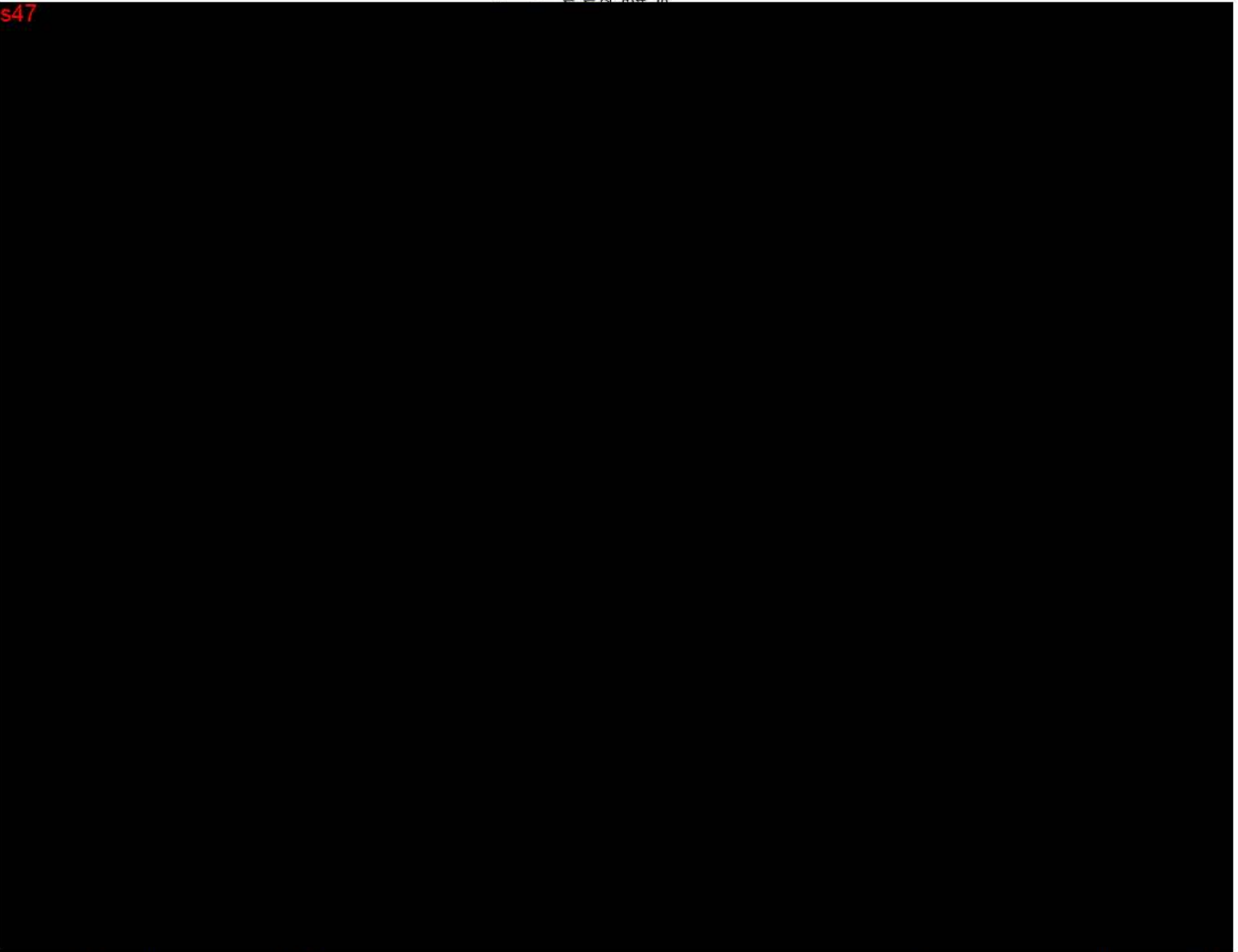


Sample: FG3716-2109003217

s22



s47



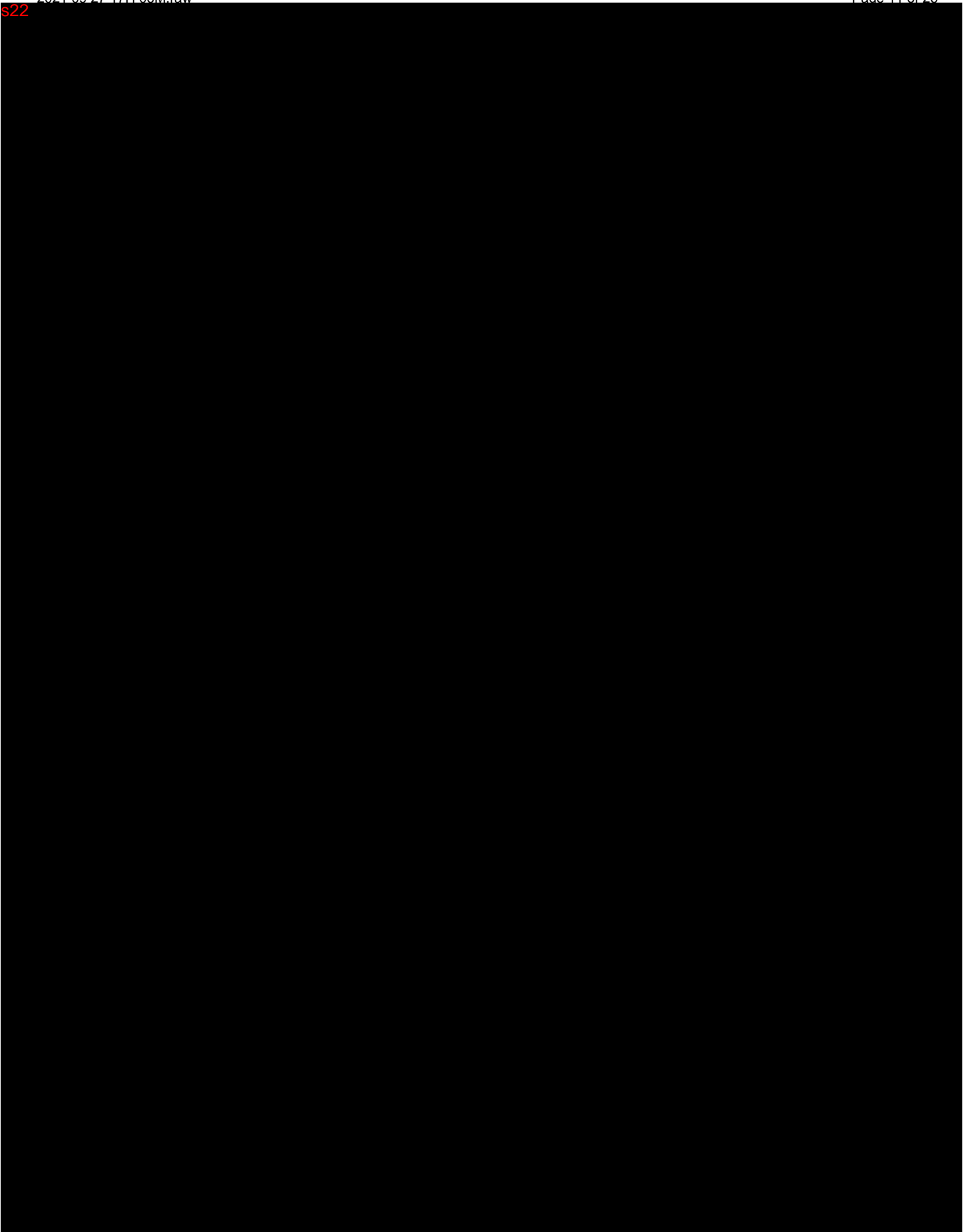
s22



s22



S22





S22

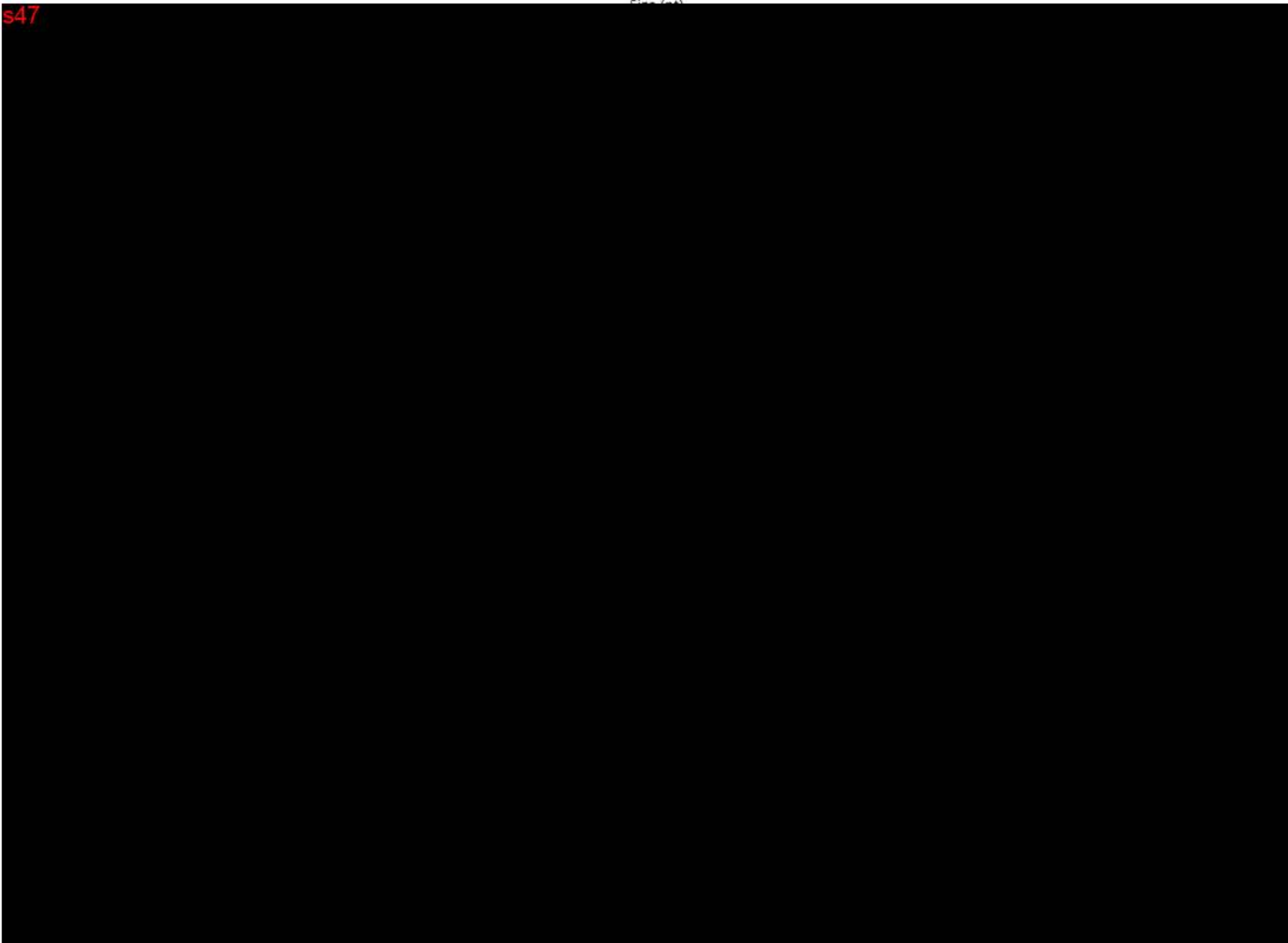
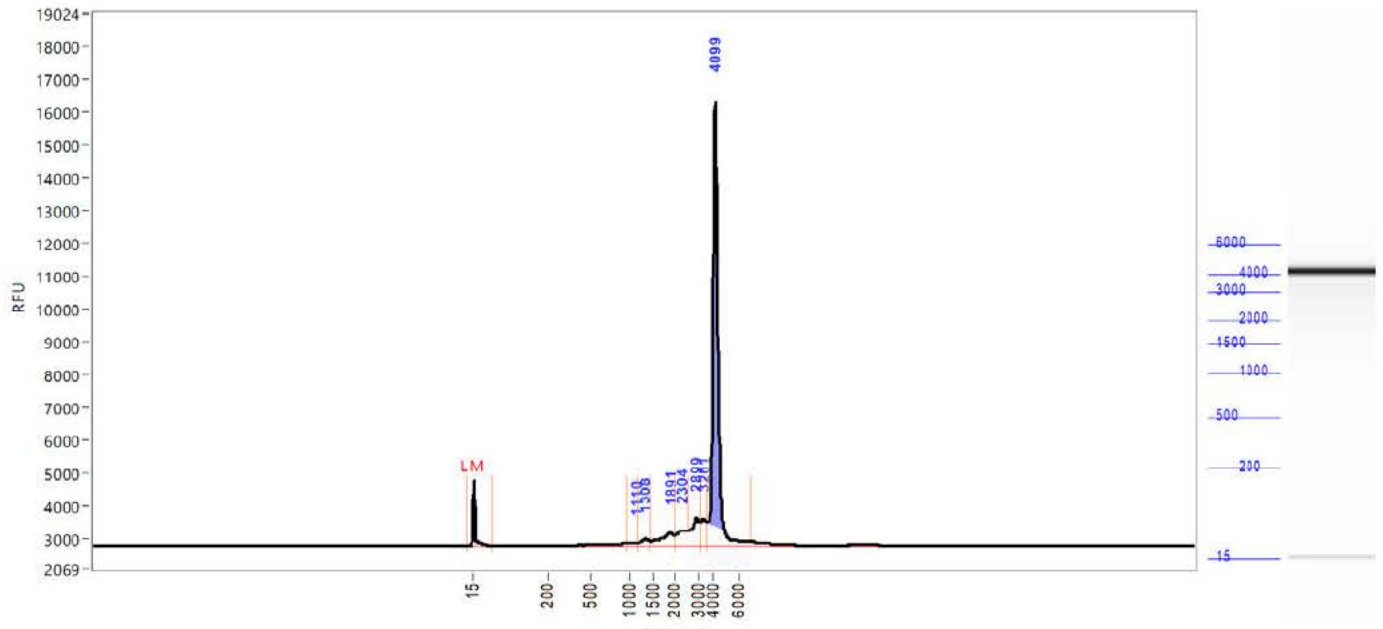


s22



Sample: EG3716-2109003217

s22



S22

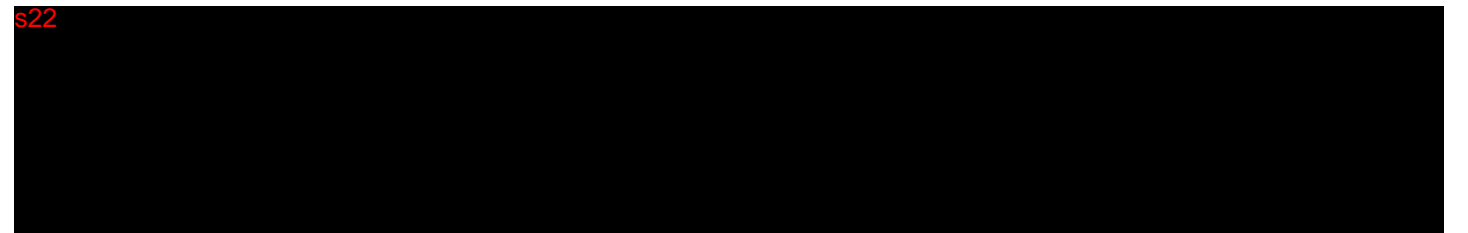


s22



s22





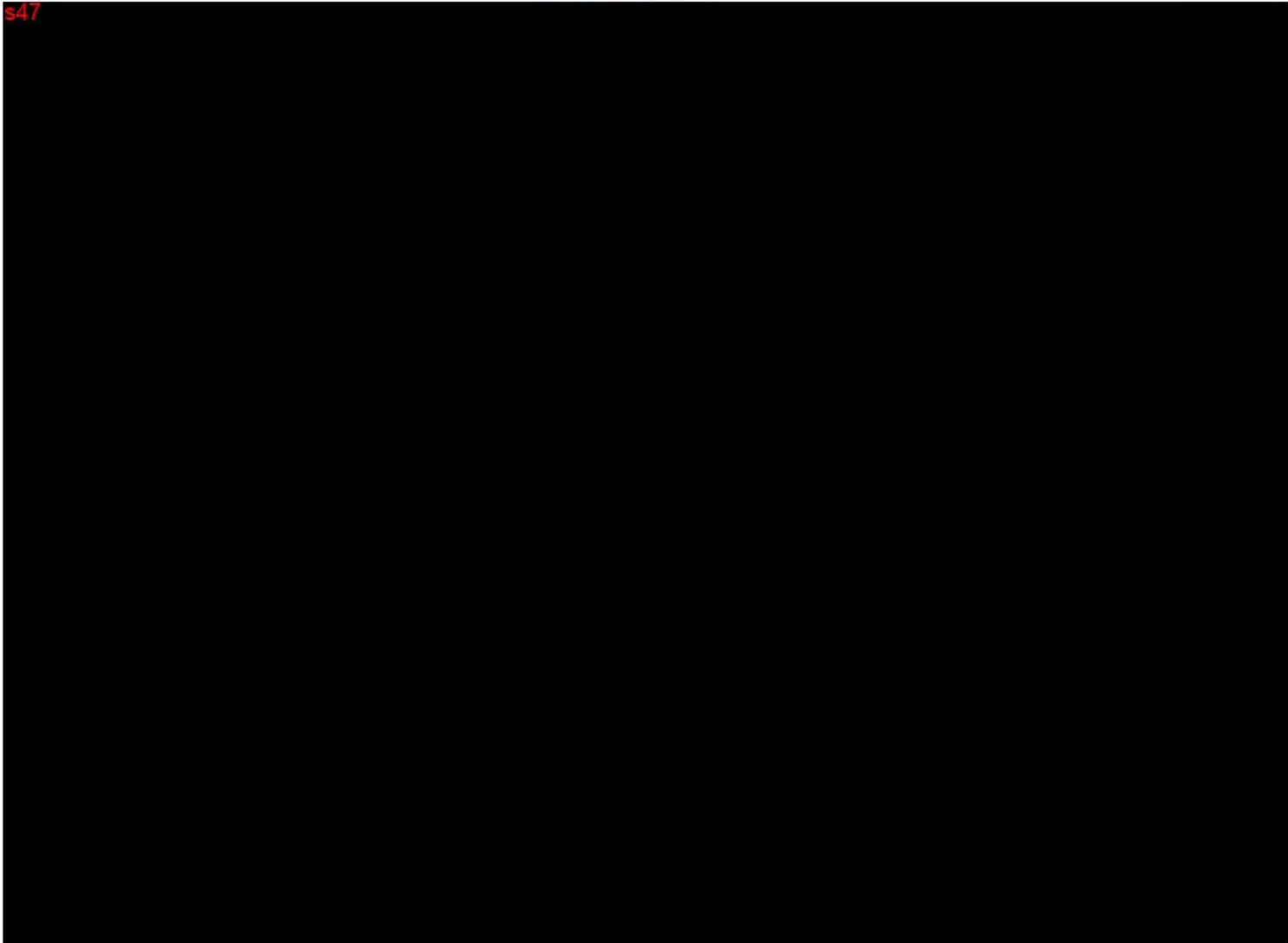
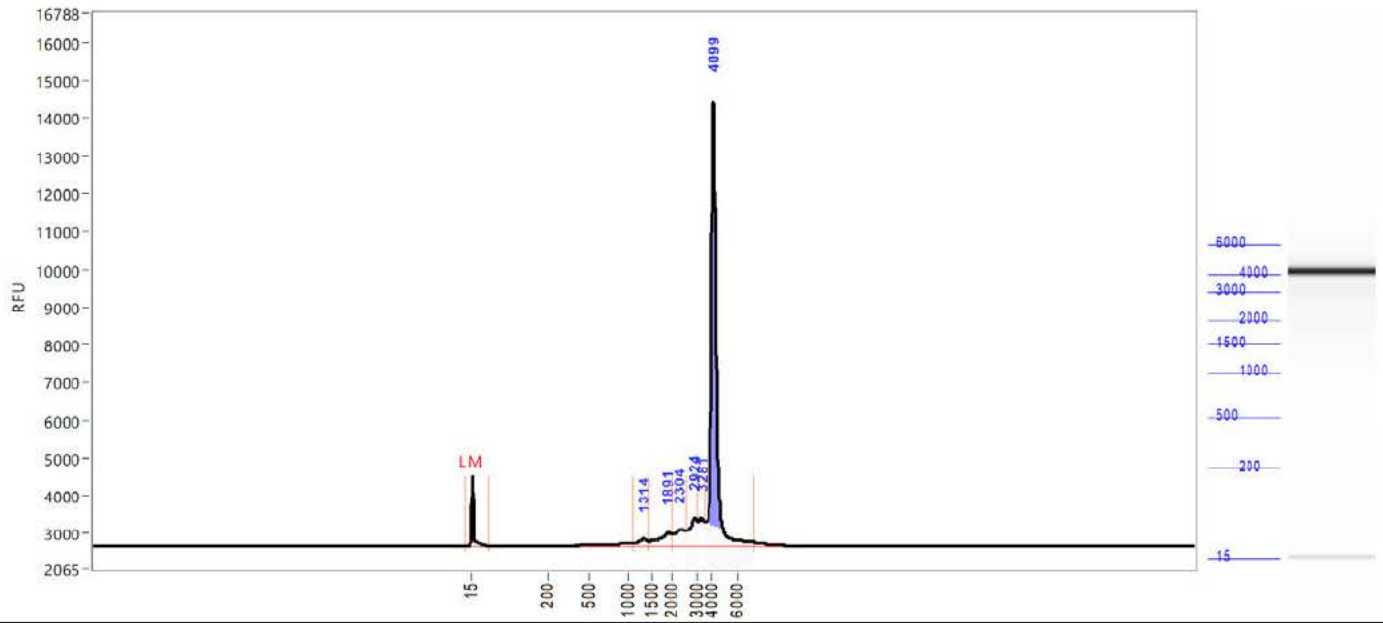
s22





Sample: FG3716-2109003217

s22



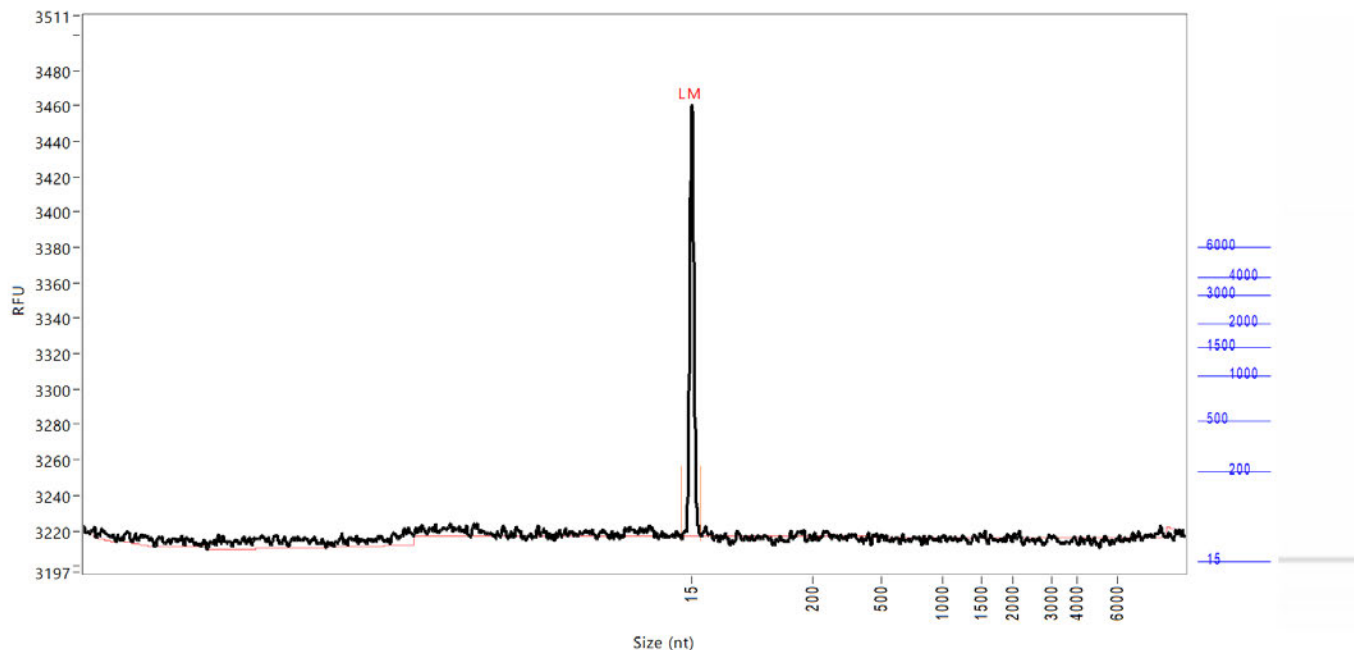
s22



s22



**Sample:** Blank  
**Well location:** D10

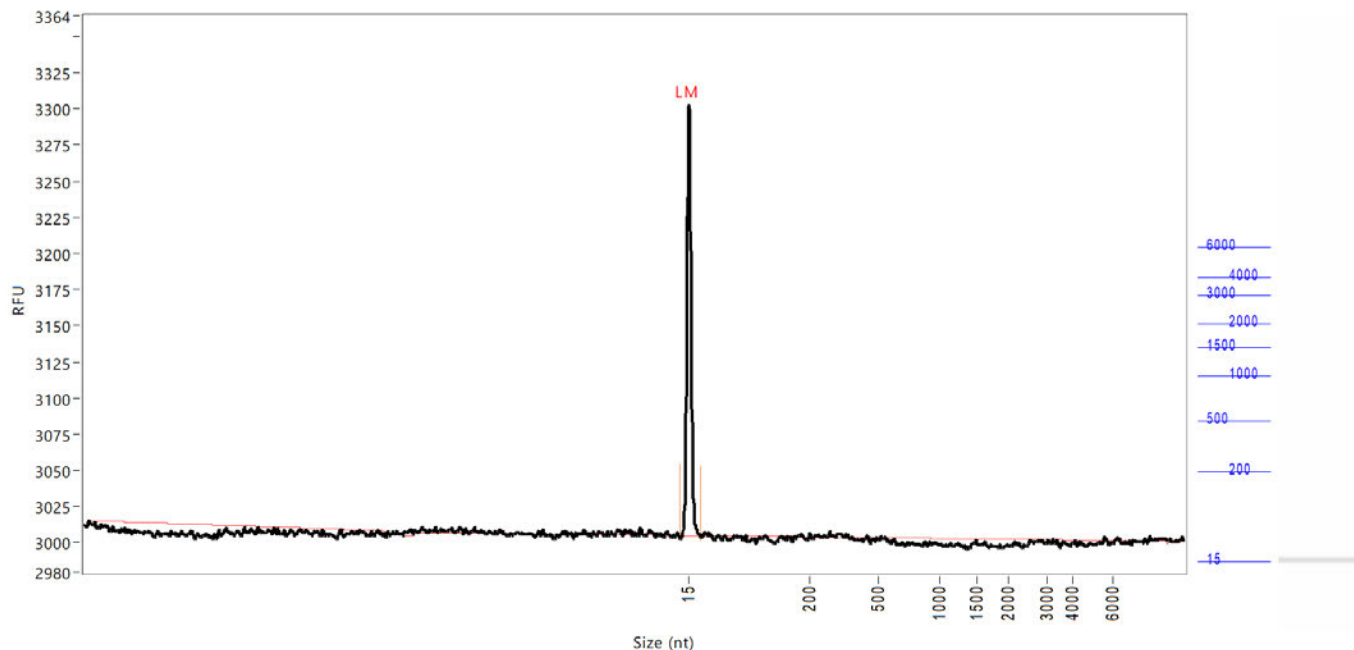


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.9661	0	29	242
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.2303	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0804 ng/ul	34.9 %Total	0.0312 nmole/L	8039 Avg. Size (nt)	4.78 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 200 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Blank  
**Well location:** D11

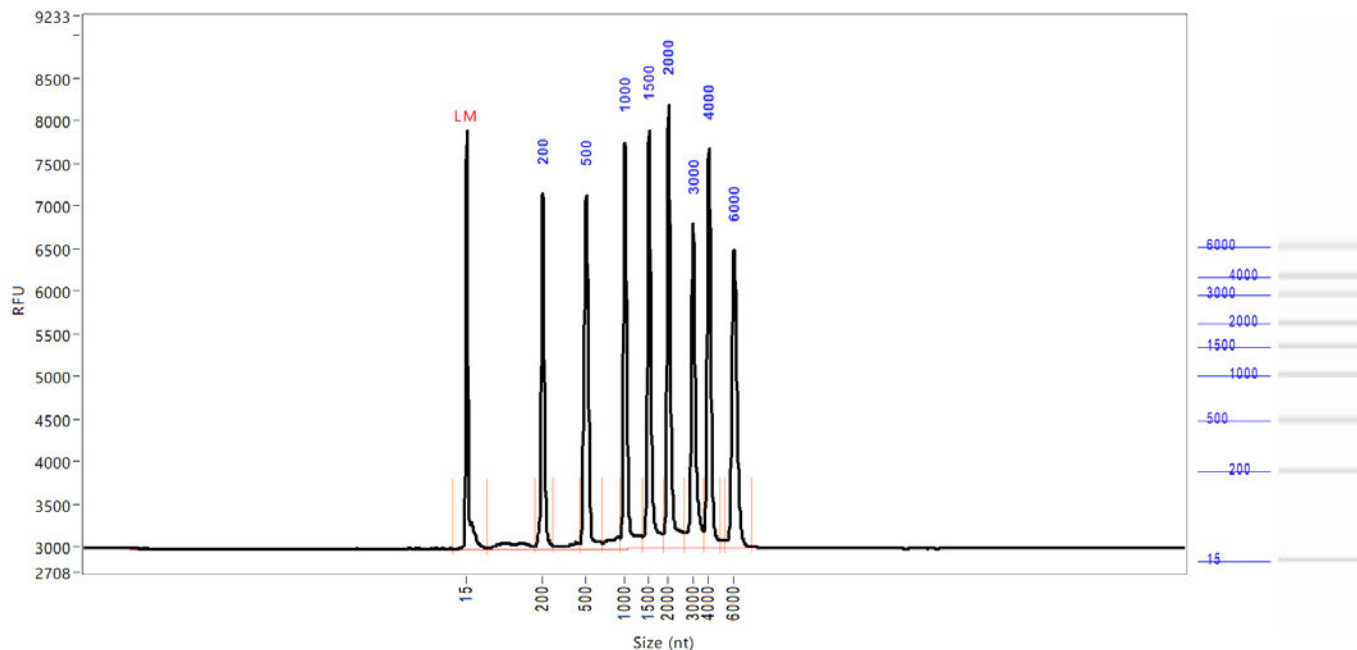


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.9661	2	34	296
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0237	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/uL	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0038 ng/uL	16.1 %Total	0.0015 nmole/L	8178 Avg. Size (nt)	5.46 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 200 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12



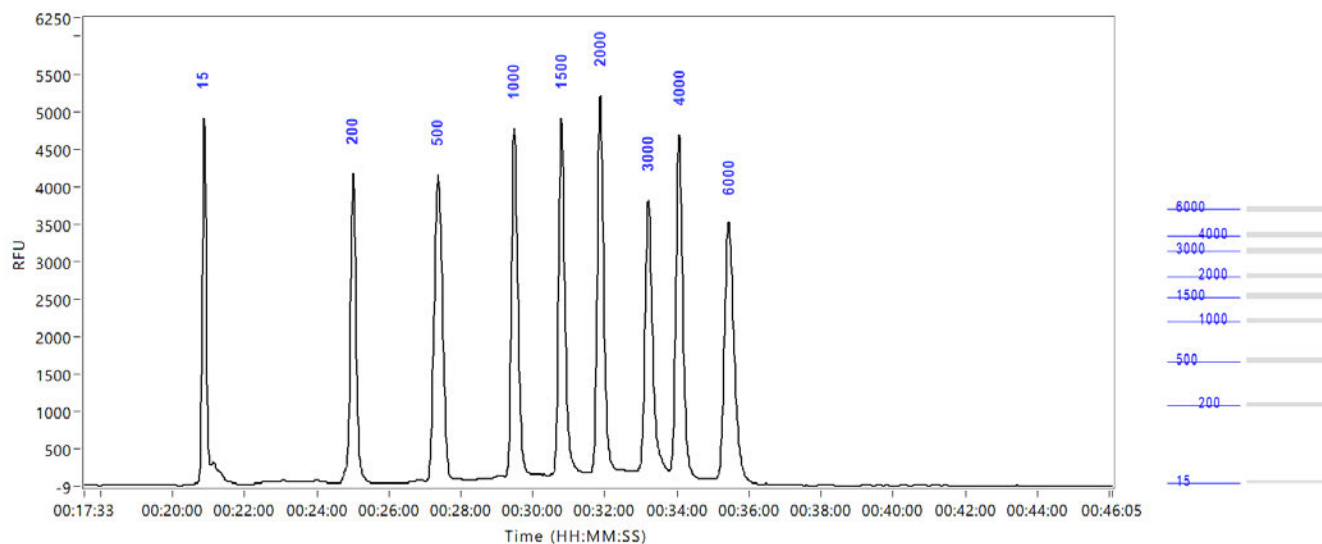
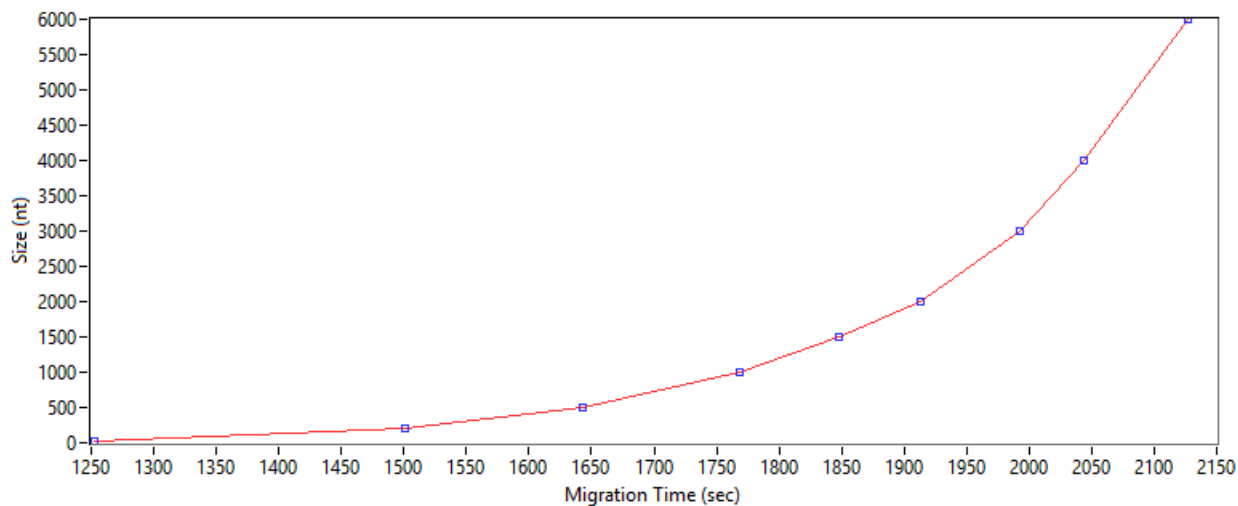
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.5000	0	64	4912
2	200	5.4948	181	277	4161
3	500	7.2024	455	713	4147
4	1000	6.4547	936	1359	4764
5	1500	6.3441	1359	1860	4908
6	2000	6.6861	1860	2621	5208
7	3000	5.5333	2621	3660	3807
8	4000	5.6160	3660	4879	4686
9	6000	5.5189	5293	7350	3504

TIC: 48.8502 ng/uL  
 TIM: 184.7389 nmole/L  
 Total concentration: 50.6023 ng/uL

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: Y    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 5    Marker min peak height: 200    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 200 RFU    Upper marker selection: Last peak > 200 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Lower Marker    Final concentration (ng/uL): 0.5000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:**  
**Fit type:** Point to point

Calibration curve





Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Appendix 1 - Fragment Analyzer Worksheet - Pfizer COMIRNATY	

### Worksheet for Fragment Analyzer - RNA Integrity

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-26	<b>Analysist</b>	s22
<b>TRIM link to data files</b>	D21-3142653,D21-3142652	<b>Test Date</b>	27/09/2021

Pipettes & Equipment	
Name	LIMS#
30-300 µL 12 channel pipette	N/A
p10 pipette	32835
p50 pipette	N/A
p100 pipette	32792
p200 pipette	N/A
Thermomixer	23660
Thermocycler	23865
P20	32891

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	N/A
Standard Sensitivity (SS) RNA kit Part 1 stored at 2-8°C	DNF-471-0500	6599311	2/02/2022
<i>Extra Blank solution</i>	<i>DNF-300-0008</i>	6595951	10/03/2022
Standard Sensitivity (SS) RNA kit Part 2 stored at -20°C (Diluent Marker & Intercalating dye)	Enter text.	Enter text.	Enter a date.
<i>Extra Diluent marker</i>	<i>DNF-369-0004</i>	6602442	7/04/2023
Standard Sensitivity (SS) RNA kit Part 3 stored at -70°C (RNA Ladder)	DNF-382-U020	0006600148	29/03/2022
Capillary conditioning solution	DNF-475-0100	6598614	22/03/2022
DEPC water	AM9961	2004017	N/A
20% Triton-X100 / 30% Ethanol solution	In house	MC1SEP21-01	1/02/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Intercalating dye	Dnf-600-u030	6603014	9/04/2022
Enter text.	Enter text.	Enter text.	Enter a date.



Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT (Drawer B)	27/09/2021	28/09/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT (Drawer M)	27/09/2021	28/09/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT (Drawer 3)	15/09/2021	29/09/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	27/09/2021	10/10/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	27/09/2021	29/09/2021  48 hours
<b>Empty waste tray and waste bottle</b> <b>Reagents can be scaled up if required – this table provides the minimum for a single run.</b> <b>Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</b> <b>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</b>  <b>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</b>			

### 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BF-25	BF-25	BF-25	BF-25	BF-25	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	BF-25	BF-25	BF-25	BF-25	BF-25	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	BF-25	BF-25	BF-25	BF-25	BF-25	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	L

**S1-6** = Samples in triplicate (a, b or c),

**RM** = Reference material

**BF-25** = Blank solution provided in kit,

**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test. Any samples not included in the test must be crossed off the plate layout, and results table below

System Suitability Criteria – RNA Ladder			
Plate location (wells)	D12		
Parameter	Limits	Results	Comments
RNA ladder profile	Visually comparable to figure 4 of SOP	ok	PASS
All peaks present	15 200 500 1000 1500 2000 3000 4000 6000 nt	ok	PASS
Peak heights	<60000 RFU	ok	PASS
Assay Acceptance Criteria – Reference Material			
Plate location (wells)	A12 B12 C12		
LIMS #	2108002914		
BATCH #	EE8493		
EXPIRY	5/02/2022		
Parameter	Limits	Results	Comments
Profile	Visually comparable to DP electropherogram in SOP	Ok/ok/ok	PASS
Migration time	Approximately comparable to profile in SOP	4099/4099/4123	PASS
Lower marker present	LM peak	Ok/ok/ok	PASS
Peak heights	5000-600000 for 2/3 replicates	11304/11030/10690	PASS
No negative peaks or baseline shifts	No significant peaks/shifts	Ok/ok/ok	PASS
Reference Material Dilutions / Calculation / Notes			
thaw date: 20/09/21 270ng/uL = 20 uL of 530 ng/uL master stock + 19 uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution			

Sample 1 Details	
Plate location (wells)	A11 B11 C11
LIMS #	2109003442
BATCH #	s22
EXPIRY	31/01/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4099/4074/4074	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	14284/14404/14347	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>opened for the first time 27/09/21, stored at 2-8C</p> <p>270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water</p> <p>90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p>

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 2 Details	
Plate location (wells)	A10 B10 C10
LIMS #	2109003217
BATCH #	FG3716
EXPIRY	30/11/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4074/4099/4099	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	12113/13550/11766	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
opened for the first time 27/09/21, stored at 2-8C 270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 3 Details	
Plate location (wells)	A9 B9 C9
LIMS #	s22
BATCH #	
EXPIRY	30/06/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
s22			

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
Taken from FF fridge, sample had been stored incorrectly for prolonged periods. 270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
	s22
Analysist	s22
Checked by	Enter text.

Sample 4 Details	
Plate location (wells)	A8 B8 C8
LIMS #	s22
BATCH #	
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
s22			

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>Taken from FF fridge, sample had been stored incorrectly for prolonged periods                      270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water                      90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>Well B8 was excluded from average calculations due to baseline shifts which interfered with the integration of peaks.</p>

Sample Results	
	s22
Analysist	s22
Checked by	Enter text.

Sample 5 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Enter text.	Enter text.	Choose an item.
Lower marker	Enter text.	Enter text.	Choose an item.
Peak heights	Enter text.	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

Sample 6 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Enter text.	Enter text.	Choose an item.
Lower marker	Enter text.	Enter text.	Choose an item.
Peak heights	Enter text.	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.



Notes

Enter text.



Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

Owner: s22	Number: Bio-BEE-Form-42
Author: s22	Version: 1
Active: 2/07/2021	Review: <QPulse_DocReviewDate>
Title: Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 27Sep2021

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899

LRW Expiry: 18 March 2022

Other Reagent: Pyrosperser Batch# 0000904583  
October 2021

Expiry: 29 June 2022 Use By: 19

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Pfizer</u> Covid Vaccine	<u>FG3716</u>	<u>30 November 2021</u>	<u>2109003217-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
<u>n/a</u>	s47	<u>2500</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of Vaccine</u>	<u>975uL</u>	<u>5uL Pyrosperser</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrosperser</u>
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>2.25</u>	s47	<u>3.24</u>	<u>183</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

### Notes:

Made a typo mistake in the report. It should be ID: 2109003217-R1 not 2109003442-R1 for this batch (B#: FG3716). s22 27Sep2021

Checked s22 28Sep2021

Data from Smear Analysis Table

1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate
2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the two smear sets.
3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	A1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A1	A1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A2	A2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A2	A2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A3	A3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A3	A3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A4	A4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A4	A4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A5	A5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A5	A5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A6	A6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A6	A6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A7	A7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A7	A7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A8	A8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A8	A8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A9	A9	Blank	3700 nt to 4800 nt	0.0035	46.7	0.0025	4480	1.22
A9	A9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A10	A10	36678TB - 22040						
A10	A10	36678TB - 22040						
B1	B1	Blank	3700 nt to 4800 nt	0.004	27.8	0.0027	4751	0.8
B1	B1	Blank	4800 nt to 13000 nt	0.0055	38.1	0.0036	4849	0.82
B2	B2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B2	B2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B3	B3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B3	B3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B4	B4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B4	B4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B5	B5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B5	B5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B6	B6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B6	B6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B7	B7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B7	B7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B8	B8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B8	B8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B9	B9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B9	B9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B10	B10	36678TB - 22040						
B10	B10	36678TB - 22040						
C1	C1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C1	C1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C2	C2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C2	C2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C3	C3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C3	C3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C4	C4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C4	C4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C5	C5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C5	C5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C6	C6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C6	C6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C7	C7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C7	C7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C8	C8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C8	C8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C9	C9	Blank	3700 nt to 4800 nt	0.0013	6.3	0.0009	4646	1.07
C9	C9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C10	C10	36678TB - 22040						
C10	C10	36678TB - 22040						
D1	D1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D1	D1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	3700 nt to 4800 nt	0.0077	0	0.0064	3732	0.54
D4	D4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D6	D6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D6	D6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D9	D9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D9	D9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D10	D10	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D10	D10	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D11	D11	Ladder	3700 nt to 4800 nt	11.5167	12.8	8.2165	4373	3.98
D11	D11	Ladder	4800 nt to 13000 nt	10.7944	12	5.2775	6381	6.05
D12	D12	Ladder		0	0	0	0	0
D12	D12	Ladder		0	0	0	0	0

Written By s22  
Authorised s22

Date Validated 10/06/2021  
Validation Due 10/06/2022

Revision no. 1  
LIMS number 33325

Validation Status Validation OVERDUE  
Analyst s22  
Assay Date 28/04/2022

Pass/Fail Parameters

minimum	cut off	maximum
<span style="color: red;">s47</span>		
result >>	<span style="color: red;">s47</span>	

										% INTEGRITY SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
1	A1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	9.27	16.05	173.21	FAIL	
2	B1	Blank	3700 nt to 4800 nt	0.004	27.8	0.0027	4751	0.8						
3	C1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	C2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	C3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	C4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	C5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	C6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	C7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	B8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	C8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A9	Blank	3700 nt to 4800 nt	0.0035	46.7	0.0025	4480	1.22	Blank	17.67	25.34	143.43	FAIL	
2	B9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	C9	Blank	3700 nt to 4800 nt	0.0013	6.3	0.0009	4646	1.07						
1	A10	36678TB - 2204001425							36678TB - 2204001425				PASS	
2	B10	36678TB - 2204001425												
3	C10	36678TB - 2204001425												
1	D1	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	D2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	D3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	D4	Blank	3700 nt to 4800 nt	0.0077	0	0.0064	3732	0.54	Blank	0.00	0.00	#DIV/0!	FAIL	
2	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	D6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	FAIL	
2	D8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
3	D9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						

										% LATE MIGRATING SPECIES SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
1	A1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	12.70	22.00	173.21		
2	B1	Blank	4800 nt to 13000 nt	0.0055	38.1	0.0036	4849	0.82						
3	C1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!		
2	B2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
3	C2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!		
2	B3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
3	C3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!		
2	B4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
3	C4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!		
2	B5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
3	C5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!		
2	B6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
3	C6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!		
2	B7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
3	C7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!		
2	B8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
3	C8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						
1	A9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!		
2	B9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN						

3	C9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN					
1	A10	36678TB - 2204001425											
2	B10	36678TB - 2204001425											
3	C10	36678TB - 2204001425											
#22													
#47													
#47													
1	D1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN					
2	D2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
3	D3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN					
1	D4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN					
2	D5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
3	D6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN					
1	D7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN					
2	D8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
3	D9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN					

This tab is only to be used if a replace needs to be excluded from the analysis.

Enter raw data directly into this table. All averages/ pass fail will be calculated automatically using the pass/fail parameters entered in the Calculations tab.

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS	
									Sample ID	Average	stdev	%CV		
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

Pass/Fail Parameters		
minimum	cut off	maximum
\$47		
result >> \$47		

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
									Sample ID	Average	stdev	%CV	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	

VALIDATION DATA

Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2
A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4
A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4
A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6
A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8
A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8
A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10
A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1
A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12
A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2
A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14
A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
A8	sample8-rep1	3500 nt to 5389 nt	40	80	82.5	4079	16
A8	sample8-rep1	5389 nt to 13000 nt	4	8	10.5	6774	1.6
A9	sample9-rep1	3500 nt to 5389 nt	45	90	92.5	4053	18
A9	sample9-rep1	5389 nt to 13000 nt	4.5	9	11.5	6916	1.8
A10	sample10-rep1	3500 nt to 5389 nt	50	100	102.5	4045	20
A10	sample10-rep1	5389 nt to 13000 nt	5	10	12.5	6870	2
A11	sample11-rep1	3500 nt to 5389 nt	55	110	112.5	4089	22
A11	sample11-rep1	5389 nt to 13000 nt	5.5	11	13.5	7320	2.2
A12	sample12-rep1	3500 nt to 5389 nt	60	120	122.5	4061	24
A12	sample12-rep1	5389 nt to 13000 nt	6	12	14.5	7135	2.4
B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62
B8	sample8-rep2	3500 nt to 5389 nt	40.5	81	83.5	4091	16.2
B8	sample8-rep2	5389 nt to 13000 nt	4.55	9	11.6	5534	1.82
B9	sample9-rep2	3500 nt to 5389 nt	45.5	91	93.5	4061	18.2
B9	sample9-rep2	5389 nt to 13000 nt	5.05	10	12.6	4079	2.02
B10	sample10-rep2	3500 nt to 5389 nt	50.5	101	103.5	4033	20.2
B10	sample10-rep2	5389 nt to 13000 nt	5.55	11	13.6	6807	2.22
B11	sample11-rep2	3500 nt to 5389 nt	55.5	111	113.5	4069	22.2
B11	sample11-rep2	5389 nt to 13000 nt	6.05	12	14.6	7000	2.42
B12	sample12-rep2	3500 nt to 5389 nt	60.5	121	123.5	4067	24.2
B12	sample12-rep2	5389 nt to 13000 nt	6.55	13	15.6	7094	2.62
C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4
C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4
C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4
C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4
C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4
C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4
C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4
C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84
C8	sample8-rep3	3500 nt to 5389 nt	41	82	84.5	4089	16.4
C8	sample8-rep3	5389 nt to 13000 nt	5.1	10	12.7	5684	2.04
C9	sample9-rep3	3500 nt to 5389 nt	46	92	94.5	4065	18.4
C9	sample9-rep3	5389 nt to 13000 nt	5.6	11	13.7	5530	2.24
C10	sample10-rep3	3500 nt to 5389 nt	51	102	104.5	4037	20.4
C10	sample10-rep3	5389 nt to 13000 nt	6.1	12	14.7	6551	2.44
C11	sample11-rep3	3500 nt to 5389 nt	56	112	114.5	4061	22.4
C11	sample11-rep3	5389 nt to 13000 nt	6.6	13	15.7	6970	2.64
C12	sample12-rep3	3500 nt to 5389 nt	61	122	124.5	4070	24.4
C12	sample12-rep3	5389 nt to 13000 nt	7.1	14	16.7	6740	2.84
D1	sample13-rep1	3500 nt to 5389 nt	65	130	15.5	4079	2.6
D1	sample13-rep1	5389 nt to 13000 nt	2.15	13	6.8	4079	0.86
D2	sample13-rep2	3500 nt to 5389 nt	65.5	131	25.5	3757	4.6
D2	sample13-rep2	5389 nt to 13000 nt	2.65	14	7.8	9444	1.06
D3	sample13-rep3	3500 nt to 5389 nt	66	132	35.5	4079	6.6
D3	sample13-rep3	5389 nt to 13000 nt	3.15	15	8.8	4079	1.26
D4	sample14-rep1	3500 nt to 5389 nt	70	140	45.5	4079	8.6
D4	sample14-rep1	5389 nt to 13000 nt	3.65	14	9.8	4079	1.46
D5	sample14-rep2	3500 nt to 5389 nt	70.5	141	55.5	5026	10.6
D5	sample14-rep2	5389 nt to 13000 nt	4.15	15	10.8	6983	1.66
D6	sample14-rep3	3500 nt to 5389 nt	71	142	65.5	5240	12.6
D6	sample14-rep3	5389 nt to 13000 nt	4.65	16	11.8	6440	1.86
D7	sample15-rep1	3500 nt to 5389 nt	75	150	75.5	5240	14.6
D7	sample15-rep1	5389 nt to 13000 nt	5.15	15	12.8	6440	2.06
D8	sample15-rep2	3500 nt to 5389 nt	75.5	151	85.5	4079	16.6
D8	sample15-rep2	5389 nt to 13000 nt	5.65	16	13.8	4079	2.26
D9	sample15-rep3	3500 nt to 5389 nt	76	152	95.5	3757	18.6
D9	sample15-rep3	5389 nt to 13000 nt	6.15	17	14.8	9444	2.46
D10	Blank-rep1	3500 nt to 5389 nt	80	160	105.5	4079	20.6
D10	Blank-rep1	5389 nt to 13000 nt	6.65	16	15.8	4079	2.66
D11	Blank2-rep1	3500 nt to 5389 nt	80.5	161	115.5	4079	22.6
D11	Blank2-rep1	5389 nt to 13000 nt	7.15	17	16.8	4079	2.86
D12	Ladder	3500 nt to 5389 nt	81	162	125.5	5026	24.6
D12	Ladder	5389 nt to 13000 nt	7.65	18	17.8	6983	3.06

Pass/Fail Parameters
minimum cut off maximum
547
result >> 847

RESULTS FOR VALIDATION DATA

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	% INTEGRITY SUMMARY				
									Sample ID	Average	stdev	%CV	
	1 A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2	sample1-rep1	11.00	1.00	9.09	FAIL
	2 B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2					
	3 C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4					
	1 A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4	sample2-rep1	21.00	1.00	4.76	FAIL
	2 B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2					
	3 C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4					
	1 A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6	sample3-rep1	31.00	1.00	3.23	FAIL
	2 B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2					
	3 C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4					
	1 A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8	sample4-rep1	41.00	1.00	2.44	FAIL
	2 B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2					
	3 C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4					
	1 A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10	sample5-rep1	51.00	1.00	1.96	FAIL
	2 B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2					
	3 C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4					
	1 A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12	sample6-rep1	61.00	1.00	1.64	PASS
	2 B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2					
	3 C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4					
	1 A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14	sample7-rep1	71.00	1.00	1.41	PASS
	2 B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2					
	3 C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4					
	1 A8	sample8-rep1	3500 nt to 5389 nt	40	80	82.5	4079	16	sample8-rep1	81.00	1.00	1.23	PASS
	2 B8	sample8-rep2	3500 nt to 5389 nt	40.5	81	83.5	4091	16.2					
	3 C8	sample8-rep3	3500 nt to 5389 nt	41	82	84.5	4089	16.4					
	1 A9	sample9-rep1	3500 nt to 5389 nt	45	90	92.5	4053	18	sample9-rep1	91.00	1.00	1.10	PASS
	2 B9	sample9-rep2	3500 nt to 5389 nt	45.5	91	93.5	4061	18.2					
	3 C9	sample9-rep3	3500 nt to 5389 nt	46	92	94.5	4065	18.4					
	1 A10	sample10-rep1	3500 nt to 5389 nt	50	100	102.5	4045	20	sample10-rep1	101.00	1.00	0.99	PASS
	2 B10	sample10-rep2	3500 nt to 5389 nt	50.5	101	103.5	4033	20.2					
	3 C10	sample10-rep3	3500 nt to 5389 nt	51	102	104.5	4037	20.4					
	1 A11	sample11-rep1	3500 nt to 5389 nt	55	110	112.5	4089	22	sample11-rep1	111.00	1.00	0.90	PASS
	2 B11	sample11-rep2	3500 nt to 5389 nt	55.5	111	113.5	4069	22.2					
	3 C11	sample11-rep3	3500 nt to 5389 nt	56	112	114.5	4061	22.4					
	1 A12	sample12-rep1	3500 nt to 5389 nt	60	120	122.5	4061	24	sample12-rep1	121.00	1.00	0.83	PASS
	2 B12	sample12-rep2	3500 nt to 5389 nt	60.5	121	123.5	4067	24.2					
	3 C12	sample12-rep3	3500 nt to 5389 nt	61	122	124.5	4070	24.4					
	1 D1	sample13-rep1											



VALIDATION DATA								
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62
3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84

RESULTS FOR VALIDATION DATA										% INTEGRITY SUMMARY			
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10	12.5	4079	2	sample1-rep1	11.0	1.0	9.1	FAIL
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11	13.5	4091	2.2					
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12	14.5	4089	2.4	sample2-rep1	21.0	1.0	4.8	FAIL
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20	22.5	4053	4					
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21	23.5	4061	4.2	sample3-rep1	31.0	1.0	3.2	FAIL
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22	24.5	4065	4.4					
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30	32.5	4045	6	sample4-rep1	41.0	1.0	2.4	FAIL
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31	33.5	4033	6.2					
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32	34.5	4037	6.4	sample5-rep1	51.0	1.0	2.0	FAIL
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40	42.5	4089	8	sample6-rep1	61.0	1.0	1.6	PASS
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41	43.5	4069	8.2					
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42	44.5	4061	8.4	sample7-rep1	71.0	1.0	1.4	PASS
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50	52.5	4061	10					
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51	53.5	4067	10.2					
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52	54.5	4070	10.4					
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60	62.5	4009	12					
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61	63.5	3998	12.2					
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62	64.5	4097	12.4					
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70	72.5	4071	14					
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71	73.5	4049	14.2					
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72	74.5	4060	14.4					

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1	3.5	6774	0.2	sample1-rep1	2.0	1.0	50.0	
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2	4.6	5534	0.42					
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3	5.7	5684	0.64	sample2-rep1	3.0	1.0	33.3	
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2	4.5	6916	0.4					
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3	5.6	4079	0.62	sample3-rep1	4.0	1.0	25.0	
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4	6.7	5530	0.84					
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3	5.5	6870	0.6	sample4-rep1	5.0	1.0	20.0	
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4	6.6	6807	0.82					
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5	7.7	6551	1.04	sample5-rep1	6.0	1.0	16.7	
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4	6.5	7320	0.8	sample6-rep1	7.0	1.0	14.3	
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5	7.6	7000	1.02					
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6	8.7	6970	1.24	sample7-rep1	8.0	1.0	12.5	
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5	7.5	7135	1					
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6	8.6	7094	1.22					
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7	9.7	6740	1.44					
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6	8.5	4079	1.2					
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7	9.6	5436	1.42					
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8	10.7	8653	1.64					
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7	9.5	7717	1.4					
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8	10.6	7570	1.62					
3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9	11.7	8404	1.84					

## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2022\_04\_28\15-58-36\2022\_04\_28\_15H\_58M.raw

**Created:** Thursday, April 28, 2022 4:24:40 PM

**Number of capillaries:** 13

**Array serial number:** 022621-27SFS

**Effect length:** 33 cm

**Array usage count:** 58

**Instrument type:** 5300 Fragment Analyzer

**Instrument controller software version:** 3.1.0.12

**Device serial number:** MY2105AB19

## Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds

**Gel prime:** No

**Full conditioning:** Yes

**Gel prime to buffer:** Yes

**Gel selection:** Gel 2

**Perform prerun:** 8.0 kV, 30 sec.

**Rinse:** No

**Marker 1:** No

**Rinse:** Tray: 3, Row: A, Dip count: 2

**Sample injection:** 5.0 kV, 6 sec.

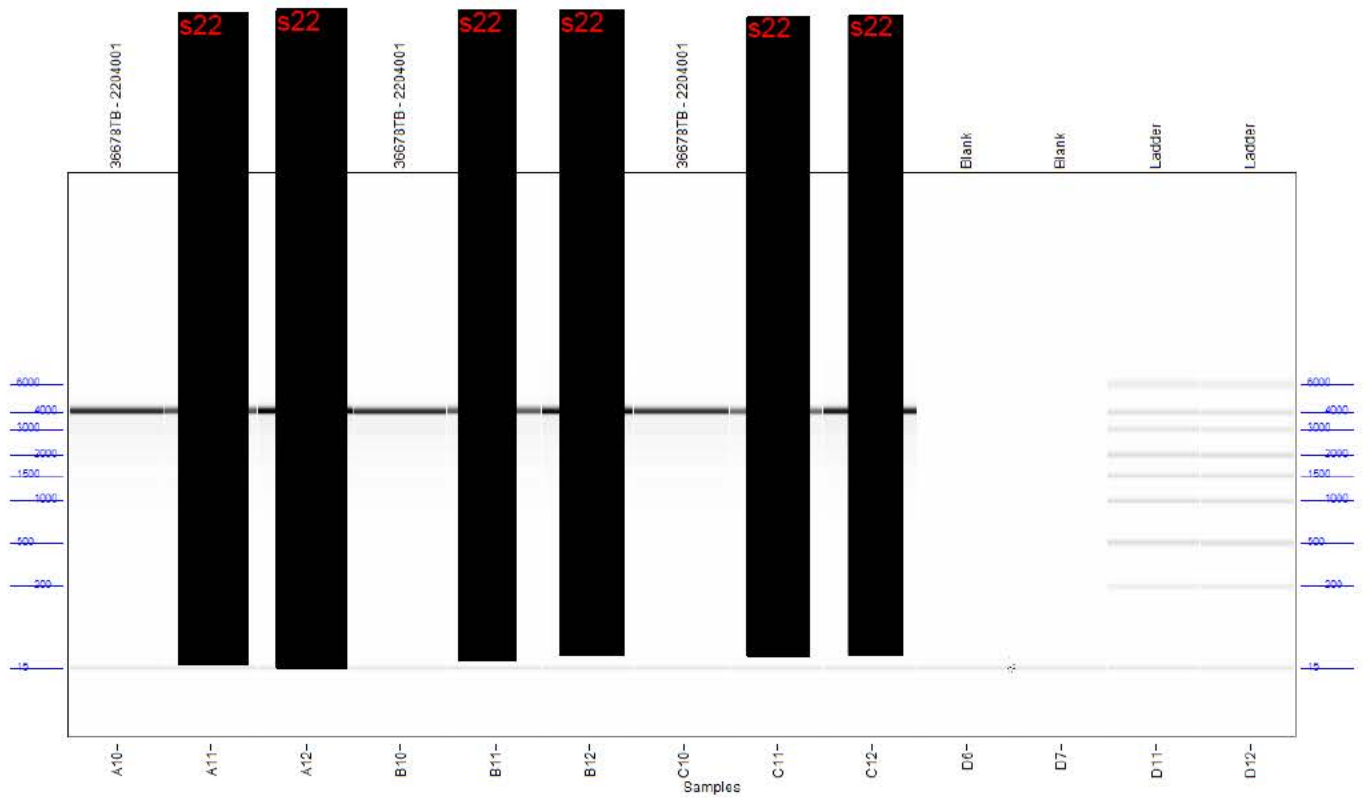
**Separation:** 8.0 kV, 60.0 min.

**Tray name:** Tray-1

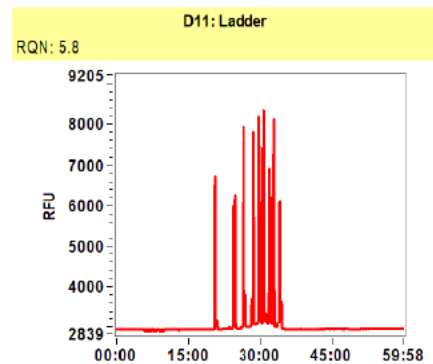
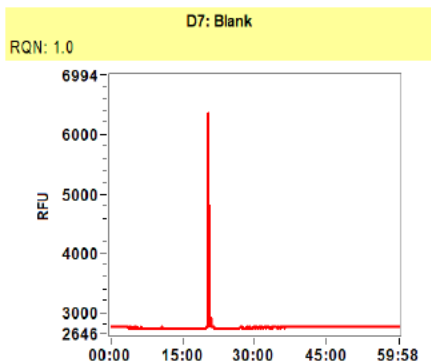
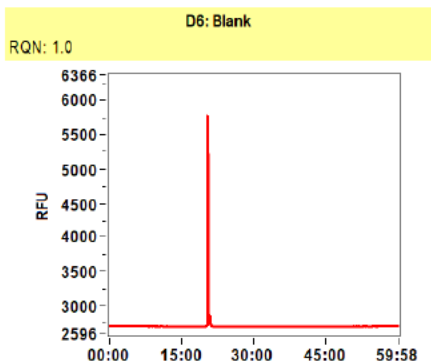
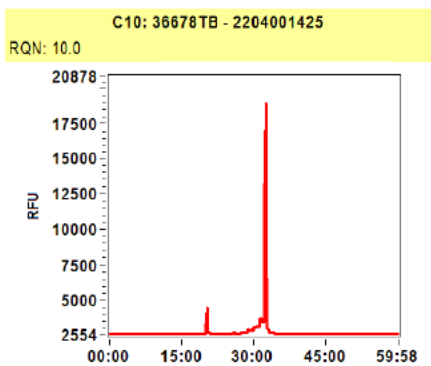
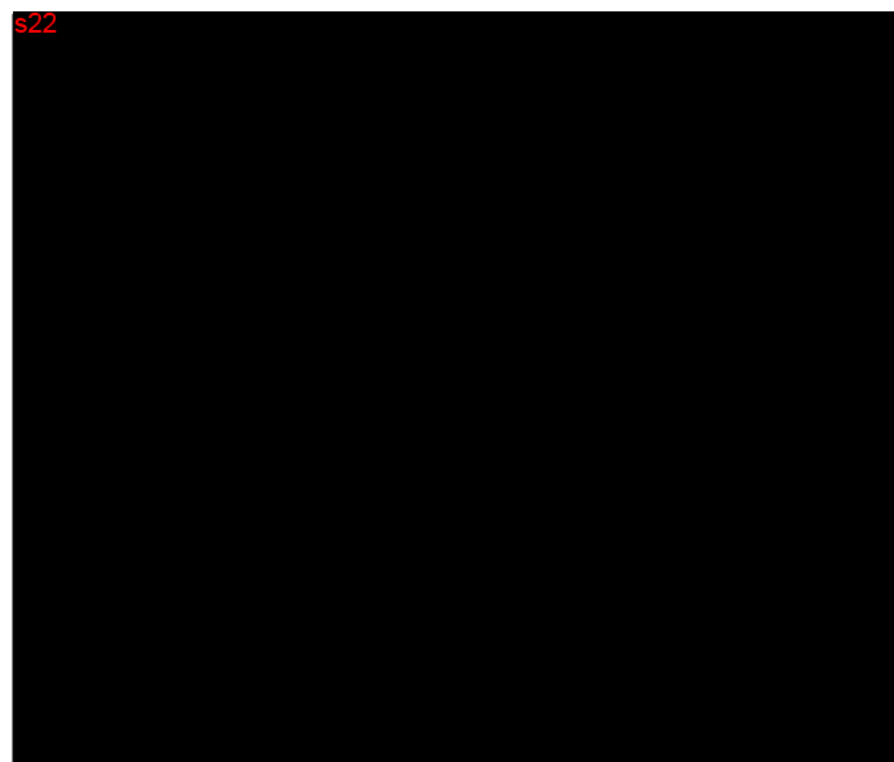
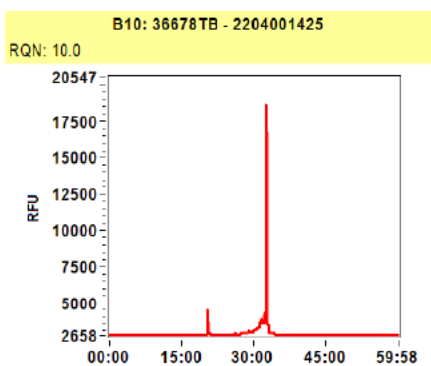
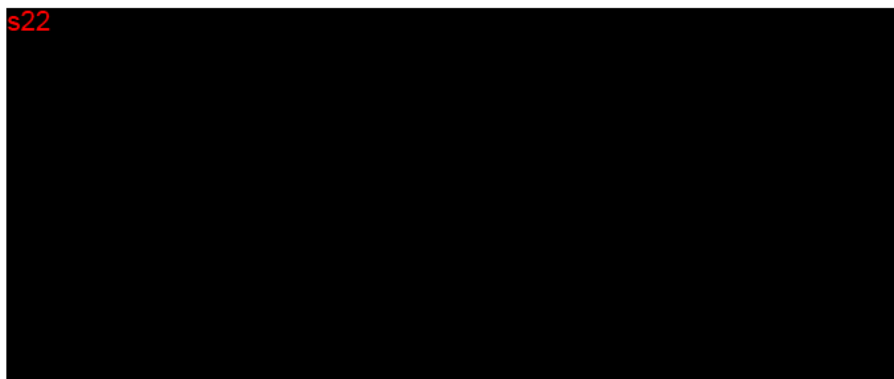
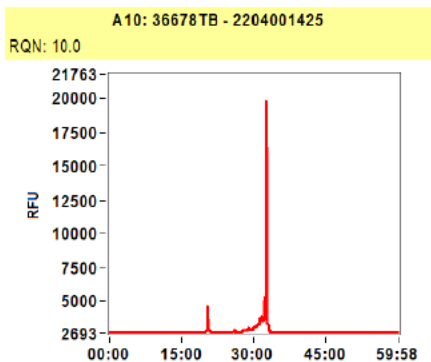
**Analysis mode:** RNA (Eukaryotic)

## Notes

### Gel Image

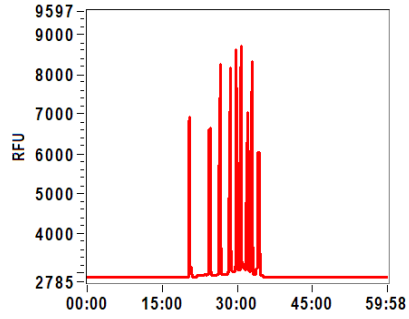


s22



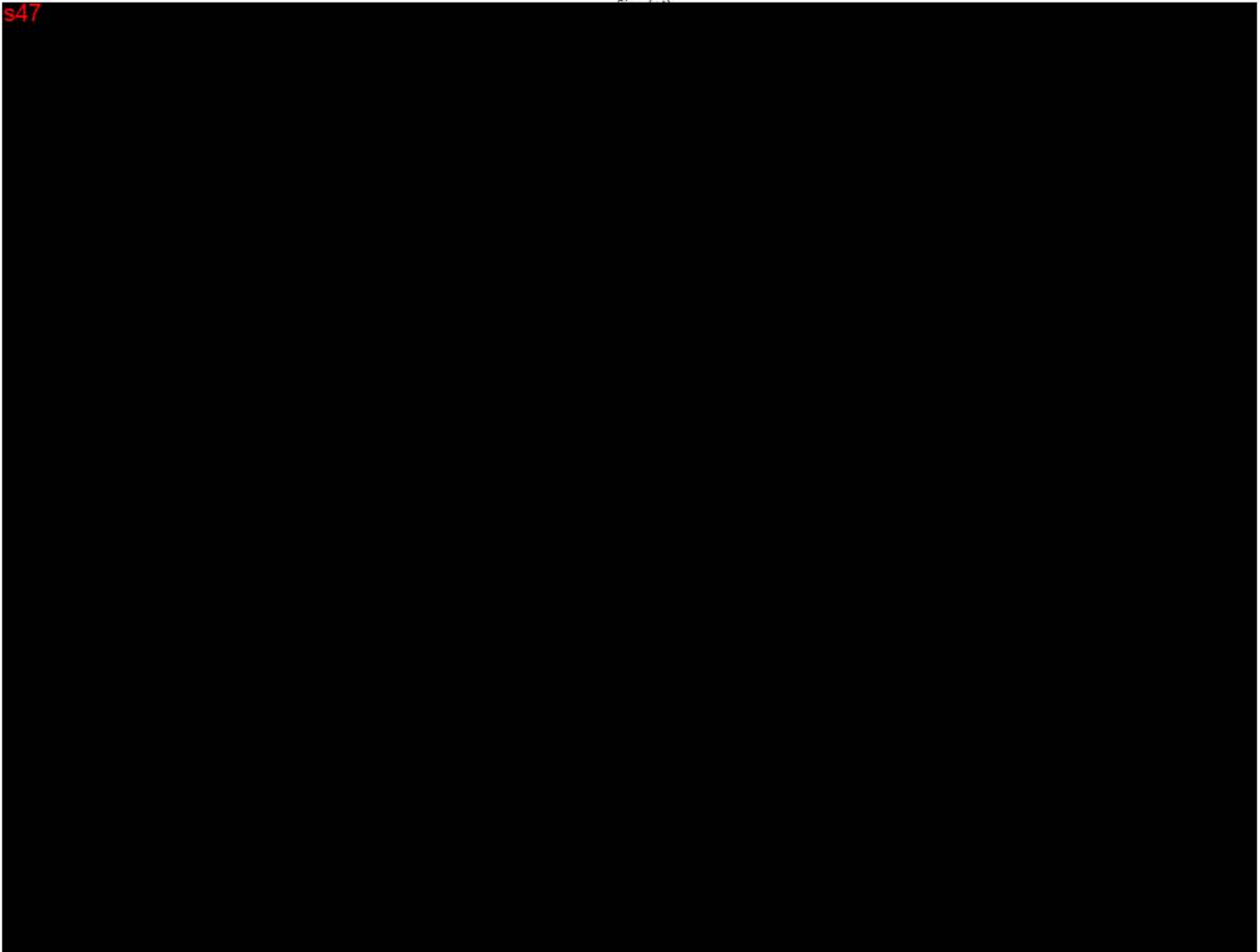
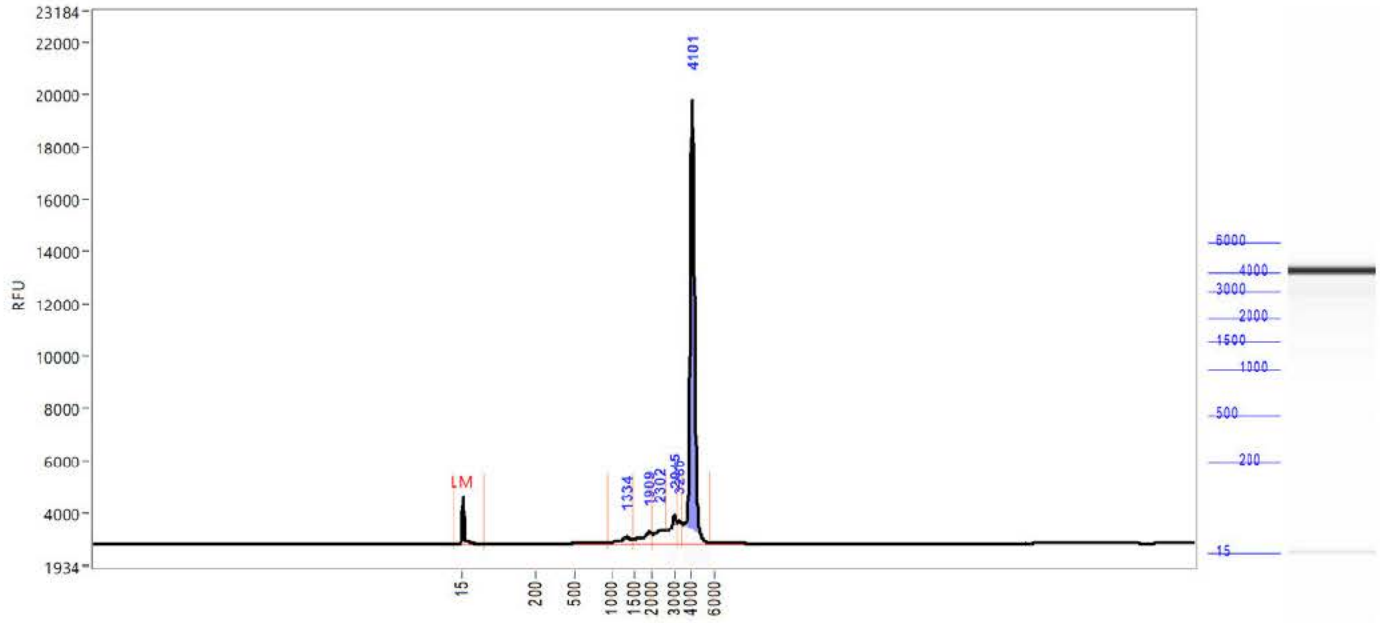
S22

D12: Ladder



Sample: 36678TB - 2204001425

s22



s47

s22



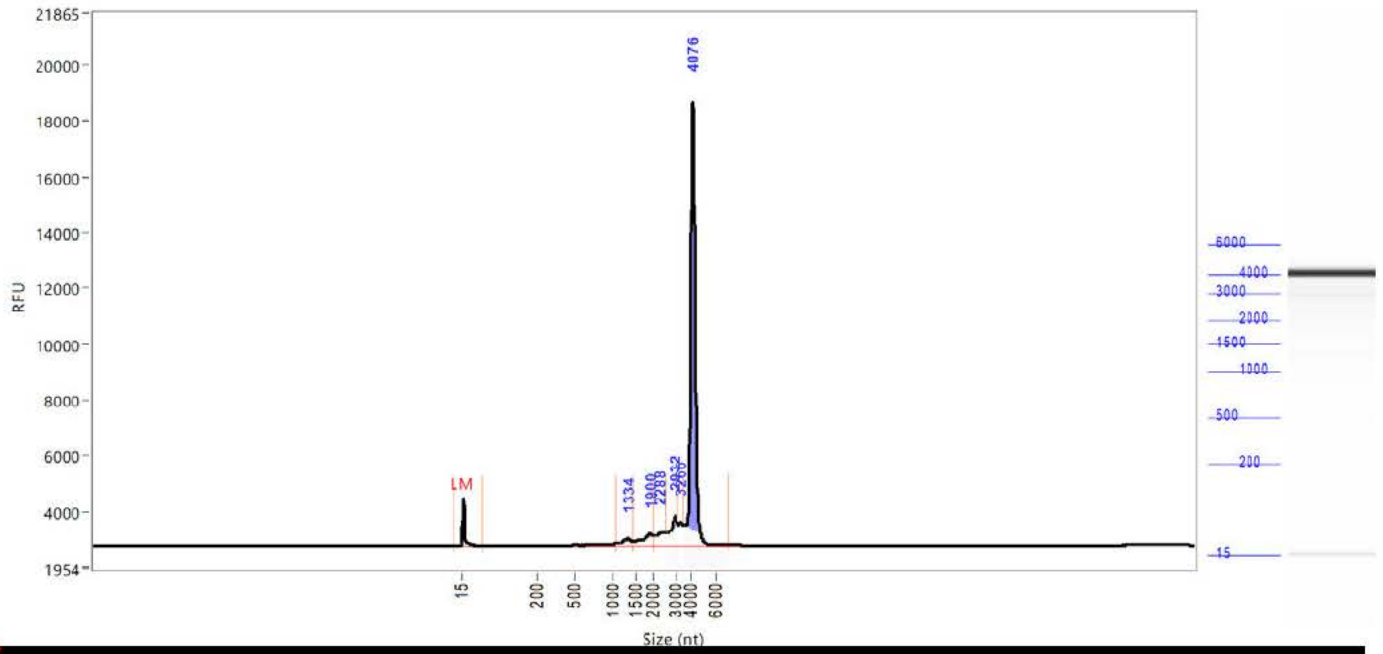
S22



Minimum RFU for data processing: 2



s22



s47

S22

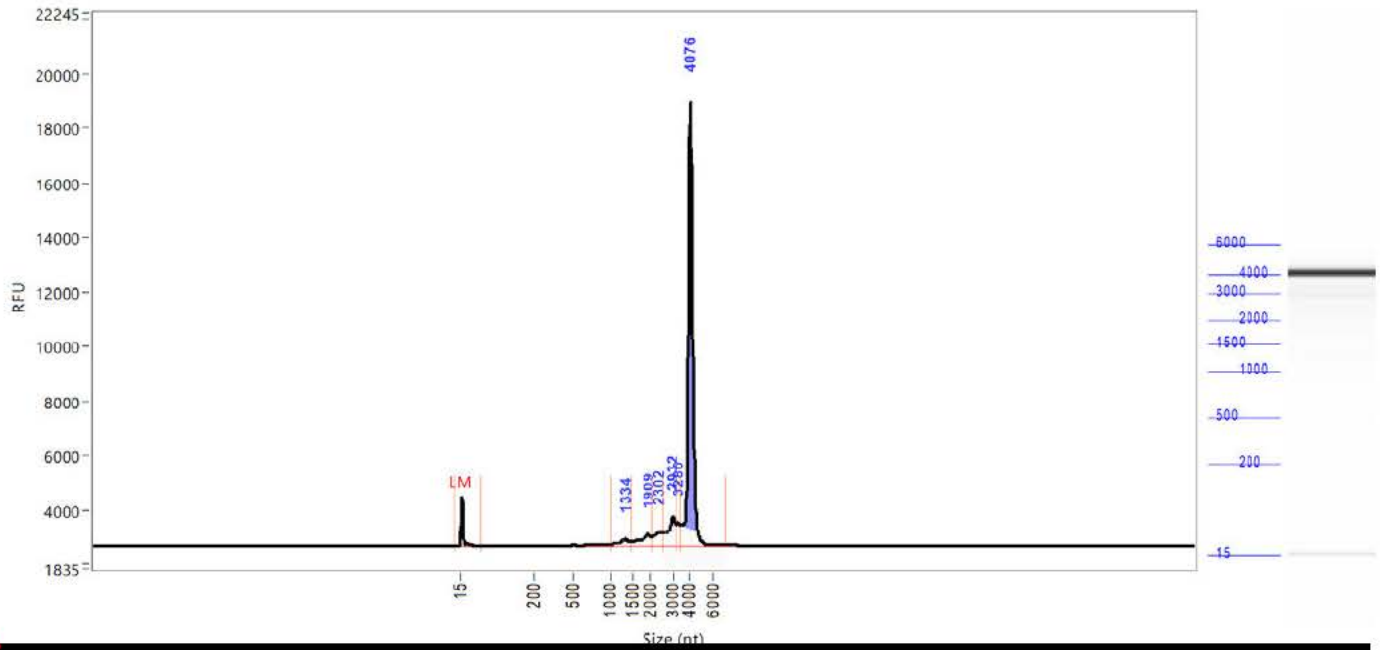


s22



Sample: 36678TB - 2204001425

s22



s47

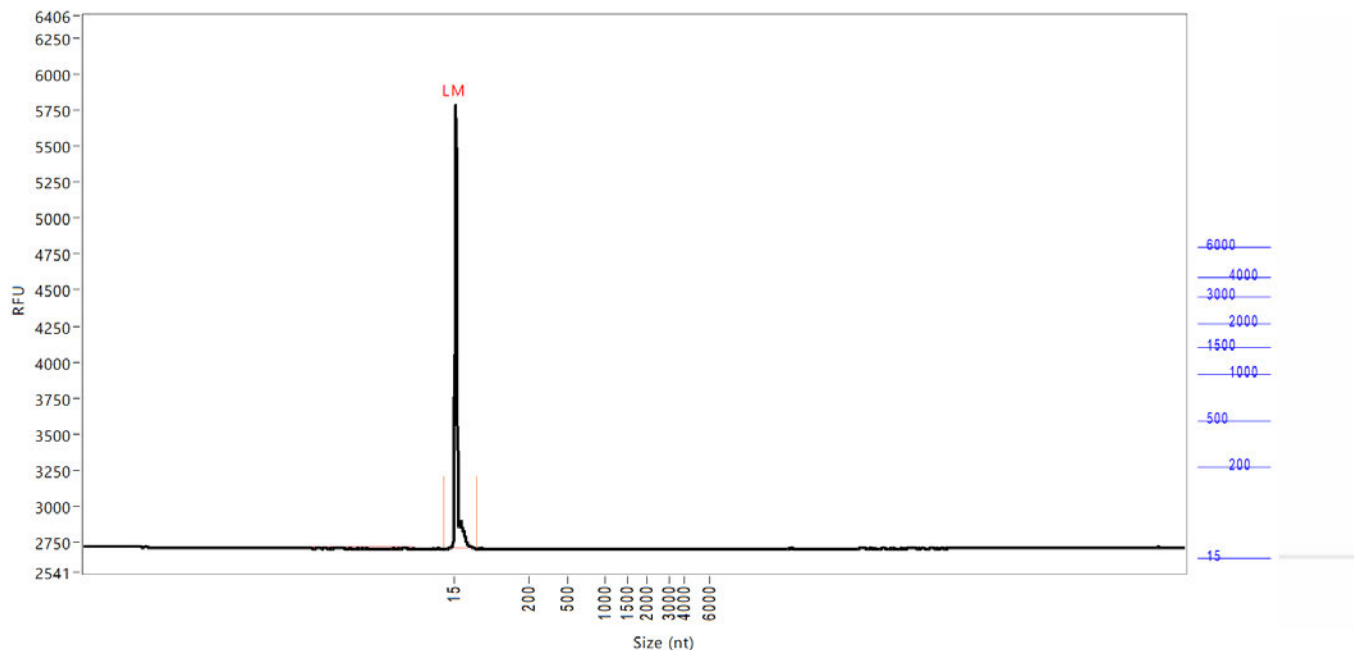
s22



s22



**Sample:** Blank  
**Well location:** D6  
**Created:** Thursday, April 28, 2022 4:24:40 PM

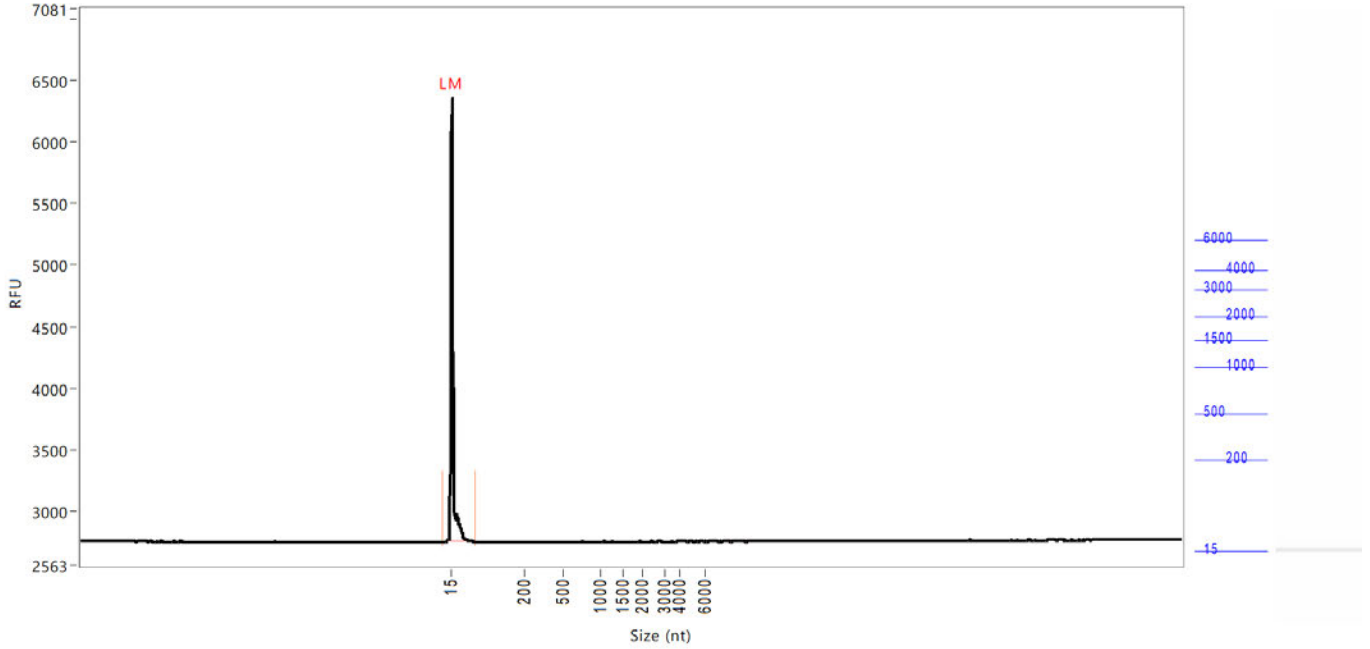


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.7196	0	67	3073
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0012	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: Y    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 0    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Blank  
**Well location:** D7  
**Created:** Thursday, April 28, 2022 4:24:40 PM



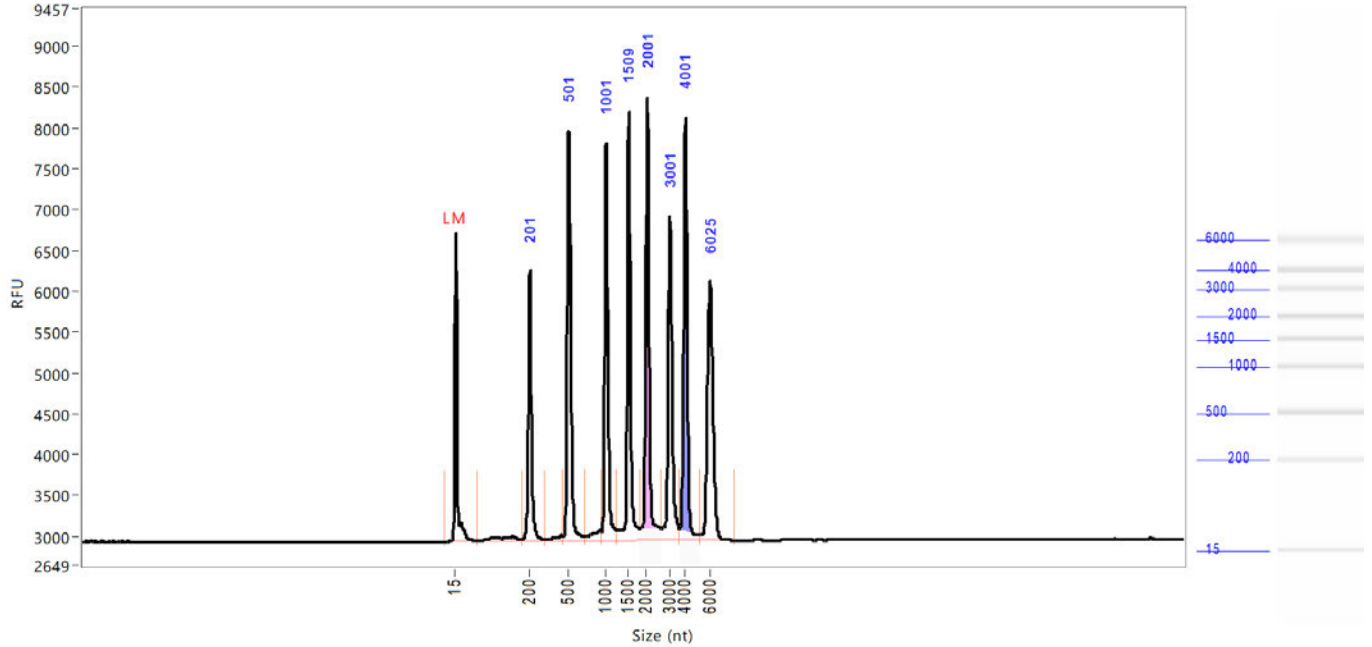
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.7196	0	75	3596
TIC:		0.0000	ng/uL		
TIM:		0.0000	nmole/L		
Total concentration:		0.0029	ng/uL		
28s/18s:		0.0			
RQN		1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: Y    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 0    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2



**Sample:** Ladder  
**Well location:** D11  
**Created:** Thursday, April 28, 2022 4:24:40 PM



Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.7196	0	69	3771
2	201	8.5129	180	311	3297
3	501	13.7835	462	714	5006
4	1001	11.3539	939	1244	4859
5	1509	11.7561	1244	1800	5234
6	2001	12.5638	1800	2631	5415
7	3001	10.2559	2631	3653	3966
8	4001	11.7301	3653	5175	5161
9	6025	10.6125	5175	8026	3162

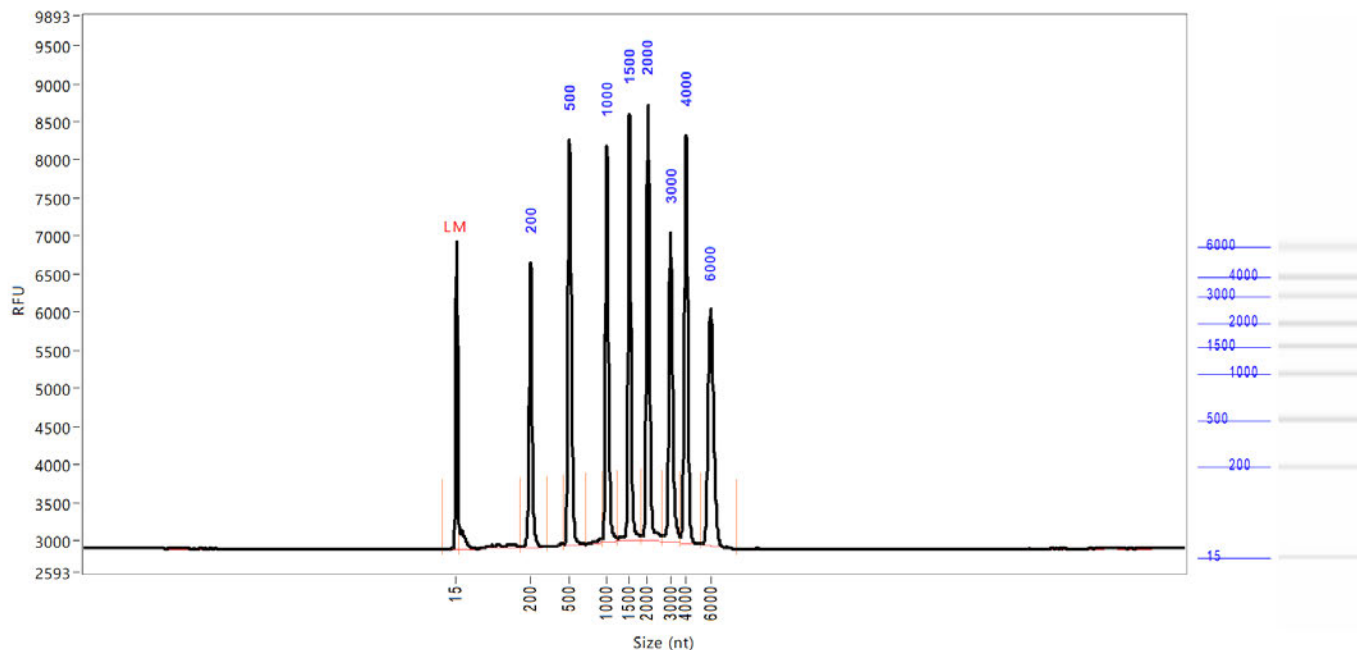
TIC: 90.5686 ng/uL  
 TIM: 317.3519 nmole/L  
 Total concentration: 92.3785 ng/uL

28s/18s: 1.0  
 RQN 5.8

Smear Analysis	3700 nt to 4800 nt	11.5167 ng/ul	12.8 %Total	8.2165 nmole/L	4373 Avg. Size (nt)	3.98 %CV
	4800 nt to 13000 nt	10.7944 ng/ul	12.0 %Total	5.2775 nmole/L	6381 Avg. Size (nt)	6.05 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: Y    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 0    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Thursday, April 28, 2022 4:24:40 PM



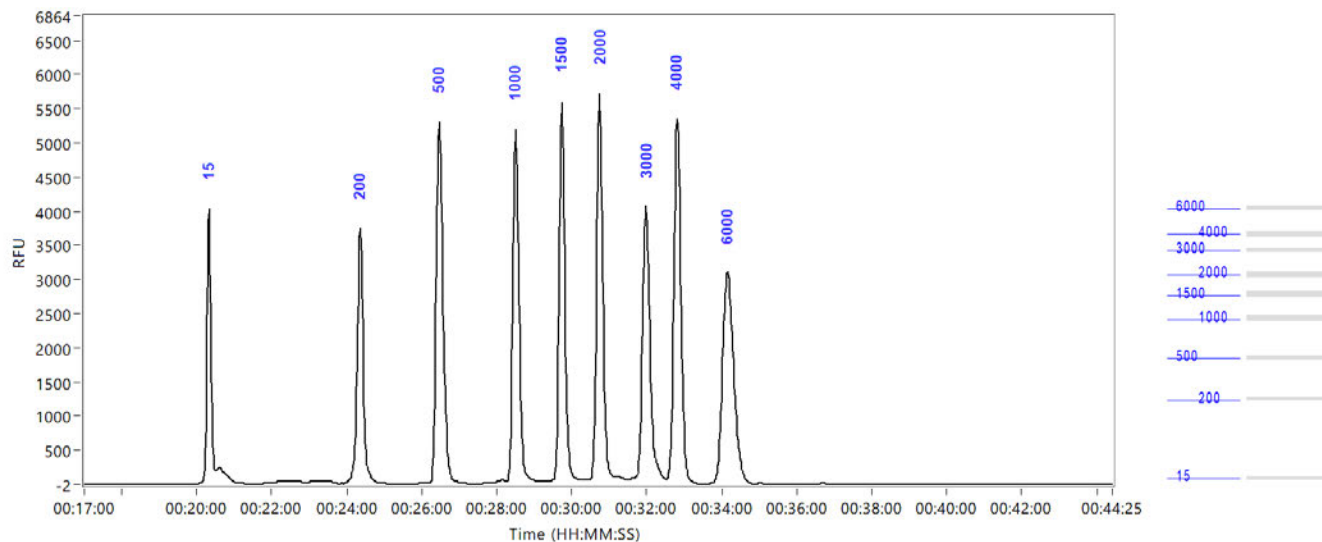
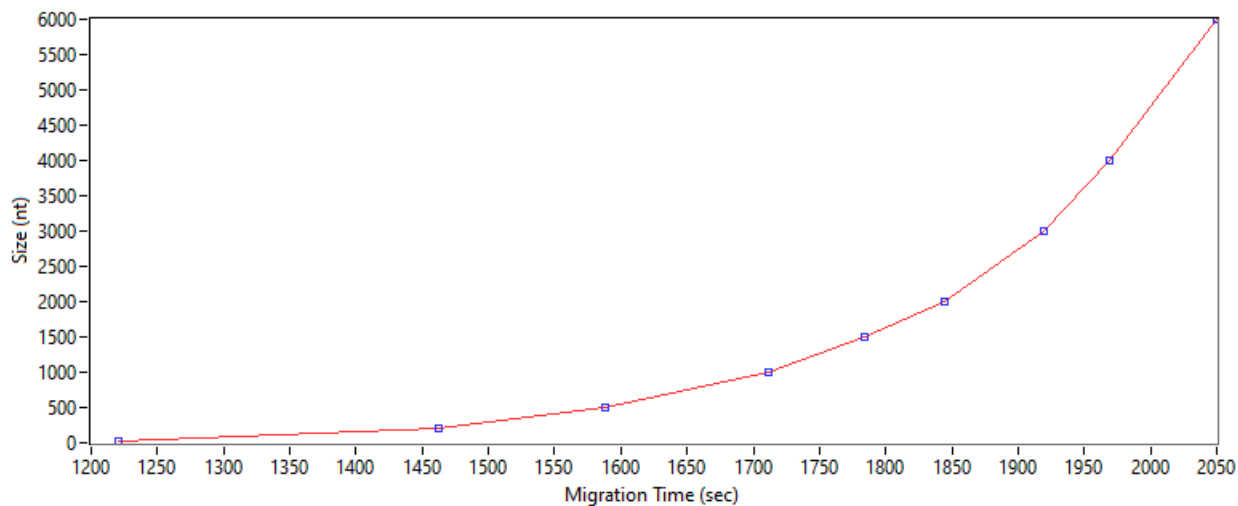
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.7196	0	23	4035
2	200	9.9408	173	330	3749
3	500	14.8540	462	718	5307
4	1000	12.1192	935	1244	5191
5	1500	12.0505	1244	1809	5595
6	2000	12.9620	1809	2631	5718
7	3000	10.5302	2631	3633	4068
8	4000	12.3165	3633	5175	5350
9	6000	10.9768	5175	8150	3101

TIC: 95.7499 ng/uL  
 TIM: 353.7722 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6      Sample min peak height: 200      Sample baseline V to V?: Y      Sample baseline V to V points: 3  
 Sample filter: Binomial      Number of points for filter: 9      Sample start region (min): 0      Sample end region (min): 60  
 Marker peak width (sec): 6      Marker min peak height: 100      Marker baseline V to V?: Y      Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU      Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder      Final concentration (ng/uL): 8.0000      Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Thursday, April 28, 2022 4:24:40 PM  
**Fit type:** Point to point

Calibration curve





<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-18
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b>	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Fragment Analyzer – Worksheet - General	

### Worksheet for Fragment Analyzer

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-31 (TRIS-Sucrose) Bio-BPC-Method-26 (PBS-Sucrose)	<b>Analyst</b>	s22
<b>TRIM link to data files</b>	D22-5425835	<b>Test Date</b>	28/04/2022
<b>Modifications to SOP</b>	1. Using a thermomixer instead of thermocycler: <a href="#">D21-3185919</a>		

Pipettes & Equipment	
Name	LIMS#
P20	Enter text.
P100	32792
P200	32892
Thermomixer	32660
P10	32833
Enter text.	Enter text.
Enter text.	Enter text.

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	N/A
Inlet Buffer	DNF-355-0300	0006623071	13/07/2022
Rinse Buffer	DNF-497-0125	000619590	29/06/2022
Capillary Storage Buffer	Enter text.	0006581286	28/12/2022
Capillary conditioning solution	DNF-475-0100	0006608461	5/05/2022
RNA Separation Gel	DNF-265-0500	0006626035	22/08/2022
Intercalating dye	DNF-600-U030	0006620194	2/07/2022
Blank	DNF-300-0008	0006615617	10/06/2022
RNA Ladder	DNF-382-U020	0006619727	30/07/2022
Diluent Marker	DNF-369-0004	0006602442	7/04/2023
DEPC water	Enter text.	2004017	N/A
20% T-X100 / 30% EtOH solution	Enter text.	SW25Mar22-01	25/06/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Enter text.	Enter text.	Enter text.	Enter a date.
Enter text.	Enter text.	Enter text.	Enter a date.

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT ( <b>Drawer B</b> )	28/04/2022	29/04/2022  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT ( <b>Drawer M</b> )	28/04/2022	29/04/2022  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT ( <b>Drawer 3</b> )	28/04/2022	11/05/2022  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	28/04/2022	29/04/2022  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	28/04/2022	30/04/2022  48 hours
<p><b>Empty waste tray and waste bottle</b></p> <p>Reagents can be scaled up if required – this table provides the minimum for a single run. Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</p> <p>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</p> <p>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</p>			

#### 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S11a	S10a	S9a	S8a	S7a	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	S11b	S10b	S9b	S8b	S7b	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	S11c	S10c	S9c	S8c	S7c	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	S12a	S12b	S12c	S13a	S13b	S13c	S14a	S14b	S14c	BF-25	BF-25	L

**S1-14** = Samples in triplicate (a, b or c), note this worksheet only contains enough fields for 6 samples.

**RM** = Reference material

**BF-25** = Blank solution provided in kit,

**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test.

System Suitability Criteria					
Tested	Well	Parameter	Limits	Results	PASS/FAIL
RNA Ladder	D12	Profile of RNA Ladder	Must be visually comparable to example given in SOP	ok	PASS
RNA Ladder	D12	All ladder peaks must be present	15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000	ok	PASS
RNA Ladder	D12	Peak heights for individual RNA ladder peaks	< 60,000 RFU	ok	PASS
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.

Assay Acceptance Criteria					
Tested RM (LIMS), blank, ladder?	Well	Parameter	Limits	Results	PASS/FAIL
RM LIMS 2111004298	A12 B12 C12	Profile	Must be visually comparable to SOP	Ok/ok/ok	PASS
RM LIMS 2111004298	A12 B12 C12	Lower marker peak	Must be present	Ok/ok/ok	PASS
RM LIMS 2111004298	A12 B12 C12	Significant negative peaks or baseline drifts	Not present	Ok/ok/ok	PASS
RM LIMS 2111004298	A12 B12 C12	Peak heights	Must be between 5000-60000 RFUs for 2/3 replicates	20759/19133/17913	PASS
RM LIMS 2111004298	A12 B12 C12	Migration time	Must be comparable to RM profile in SOP	Ok/ok/ok	PASS
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Dilutions / Calculation / Notes					
Reference Material Preparation:					
Working stock 1: 270 ng/uL = 20 x 470 ng/uL + 15 uL DEPC					
Working stock 2: 90 ng/uL = 20 x 270 ng/uL + 40 uL Triton/ethanol mix					

Sample 1					
Plate location (wells)	A11 B11 C11				
LIMS #	2204001335				
BATCH #	s22				
EXPIRY	30/09/2022				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Peak height of main peak	5000-60000 RFU	14888/14347/13882			PASS
Lower Marker peak	Must be present	Ok/ok/ok			PASS
Migration time of main peak	Must be comparable to RM	Ok/ok/ok			PASS
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Main peak	s47	s22			PASS
LMS	N/A				PASS
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
PASS					
Analysist			s22		
Checked by			s22		
Sample Dilutions / Calculation / Notes					
Working stock 1: 270 ng/uL = 20 x 500 ng/uL + 17 uL DEPC					
Working stock 2: 90 ng/uL = 20 x 270 ng/uL + 40 uL Triton/ethanol mix					



Sample 2					
Plate location (wells)	A10 B10 C10				
LIMS #	2204001425				
BATCH #	36678TB				
EXPIRY	31/08/2022				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Peak height of main peak	5000-60000 RFU	16969/15899/16307			PASS
Lower Marker peak	Must be present	Ok/ok/ok			PASS
Migration time of main peak	Must be comparable to RM	Ok/ok/ok			PASS
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
MAIN PEAK	s47	s47			PASS
LMS	N/A				PASS
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
PASS					
Analysist			s22		
Checked by			s22		
Sample Dilutions / Calculation / Notes					
Working stock 1: 270 ng/uL = 20 x 500 ng/uL + 17 uL DEPC					
Working stock 2: 90 ng/uL = 20 x 270 ng/uL + 40 uL Triton/ethanol mix					

Sample 3					
Plate location (wells)	Choose an item.				
LIMS #	Click or tap here to enter text.				
BATCH #	Click or tap here to enter text.				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
Choose an item.					
Analysist			Enter text.		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
Enter text.					

Sample 4					
Plate location (wells)	Choose an item.				
LIMS #	Click or tap here to enter text.				
BATCH #	Click or tap here to enter text.				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results	Comments		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
Choose an item.					
Analysist			Enter text.		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
Enter text.					

Sample 5					
Plate location (wells)	Choose an item.				
LIMS #	Click or tap here to enter text.				
BATCH #	Click or tap here to enter text.				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
Choose an item.					
Analysist			Enter text.		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
Enter text.					

Sample 6					
Plate location (wells)	Choose an item.				
LIMS #	Click or tap here to enter text.				
BATCH #	Click or tap here to enter text.				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results	Comments		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
Choose an item.					
Analysist			Enter text.		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
Enter text.					

**Notes**

Enter text.



<b>Type:</b> Biotherapeutics\BEE\Forms	<b>Number:</b> Bio-BEE-Form-39 / <b>Version:</b> 2
<b>Owner:</b> s22	<b>Approver:</b> s22
<b>Active:</b> 19/04/2022	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Endotoxin Routine Assay Worksheet	

## Endotoxin Routine Assay Worksheet

Assay ID: 28Apr2022 Operator: s22

### Limulus Amoebocyte Lysate (LAL)

Lysate batch and expiry recorded on software for each assay

Ensure sensitivity of LAL batch has been confirmed. 'Lysate preparation details' shown below

### Recombinant Factor C (rFC)

rFC Enzyme, Fluorogenic Substrate & rFC Assay Buffer batches and expiry dates recorded on software for each assay

Ensure sensitivity of the rFC batch has been confirmed. 'rFC Reagent preparation details' shown below

### Control Standard Endotoxin (CSE) – refer to Method 5 and form 37

CSE batch and expiry recorded on software for each assay

Reconstitution details for either KLAL or rFC – see Trim File D22-5400427

CSE Lot Number: 0000953052 Conc 50 EU/mL

### LAL Reagent Water (LRW) Lot Number: 0000966190 Expiry: 20Jul2023

How many samples were linked to this assay? 6

**This form is used for recording the assay details and results and only gives the method in point form. See SOP 28 and appropriate method for the detailed procedure.**

To avoid endotoxin contamination, use careful technique and **pyrogen free** equipment.

### Preparation of Assay

- Fill out the appropriate forms from the Quality Management System (QMS)
- Retrieve the required kit reagents from cold storage to equilibrate to room temperature
- Turn on plate reader and computer and follow the steps as detailed in Method 4

### Preparation of CSE (refer to SOP 28 and appropriate Method)

- Prepare CSE as detailed in Method 5. CSE dilutions can be dispensed to the plate as they are prepared to save mixing time

- CSE is set up as in the table below - record %CV results from the final report

#### For a KLAL (KQCL) assay

Concentration	Plate wells ID	% CV
50 EU/ml	F1 – F2	1.57
5 EU/ml	E1 – E2	1.96
0.5 EU/ml	D1 – D2	2.47
0.05 EU/ml	C1 – C2	1.72
0.005 EU/ml	B1 – B2	3.50
Blank	A1 – A2	

#### For an rFC assay

Concentration	Plate wells ID	% CV
5 EU/ml	E1 – E2	N/A
0.5 EU/ml	D1 – D2	N/A
0.05 EU/ml	C1 – C2	N/A
0.005 EU/ml	B1 – B2	N/A
Blank	A1 – A2	

- Dispense 100 µl of the appropriate dilution of CSE into the appropriate wells of the plate.
- Continue with procedure as per the appropriate method

### Preparation of Samples (refer to SOP 28 and appropriate Method)

- Prepare sample dilutions as in form 42
- Pipette 10 µl of the 5 EU/ml standard to the appropriate PPC wells as per the plate layout
- Dispense 100 µl of the final sample dilutions into the 4 appropriate wells as per the plate layout

### Starting the Assay

- The plate is then ready for the reaction. Prepare the software as set out in the Method 4
- “Run” the Template prepared earlier. Follow the prompts to the Pre-warming step

If performing a KLAL assay

- Prepare the required lysate vial/s (Method 6) and pour into the reagent reservoir

Lysate Preparations Details -



Lysate Lot Number	XL001W25CF	Expiry 04Jul2023
Date sensitivity confirmed	06Jan2022	N/A
Reconstitute lysate with	2.6mL or (3x 867uL)	mL of LAL Reagent Water (LRW)
Date reconstituted	28Apr2022	Combined with vial that reconstituted on 21/04/2022, use by 04/05/2022 (420uL)
Operator(s)	s22	N/A
Use by date	11May2022	(Lonza KQCL- 14 days from reconstitution, at below -10°C)

**OR**

*If performing an rFC assay*

- Make up the required volume of rFC reagent directly into the reservoir (Method 6)

rFC Reagent Preparations Details – (Note: once prepared, working reagent cannot be stored)

	Lot	Expiry	Volume
Date sensitivity confirmed	<u>N/A</u>	<u>N/A</u>	
Fluorogenic Substrate	<u>N/A</u>	<u>N/A</u>	<u>N/A</u> µL
rFC Assay Buffer	<u>N/A</u>	<u>N/A</u>	<u>N/A</u> µL
rFC Enzyme Solution	<u>N/A</u>	<u>N/A</u>	<u>N/A</u> µL

- Open cover – if using the Spectramax use the software to open and close the drawer
- Add 100 µl of either lysate (KLAL) **OR** working reagent (rFC) to each of the assay wells, carefully, and as quickly as possible
- Close the drawer on the plate reader and click OK to start the run. **Do not open drawer**

### Acceptance Criteria – for KLAL

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>-1.00</u>
Slope between -0.400 and -0.100	<u>-0.219</u>
Y intercept between 2.500 and 3.500	<u>3.195</u>
Mean reaction times of blank $\geq$ mean reaction times of lowest standard	<u>Yes</u>
Coefficient of variation (CV) values for all standards are $< 10\%$	<u>Yes</u>
Were all acceptance criteria for the standard curve met?	<u>Yes</u>

## Acceptance Criteria – for rFC

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>N/A</u>
Slope between 0.760 and 1.110	<u>N/A</u>
Y intercept between 2.500 and 5.000	<u>N/A</u>
Mean RFU of blank $\leq$ mean RFU of lowest standard	<u>N/A</u>
Coefficient of variation (CV) values for all standards are $< 25\%$	<u>N/A</u>
Were all acceptance criteria for the standard curve met?	<u>N/A</u>

## Conclusions

Follow procedures for 'Recording Results' detailed in Method 7

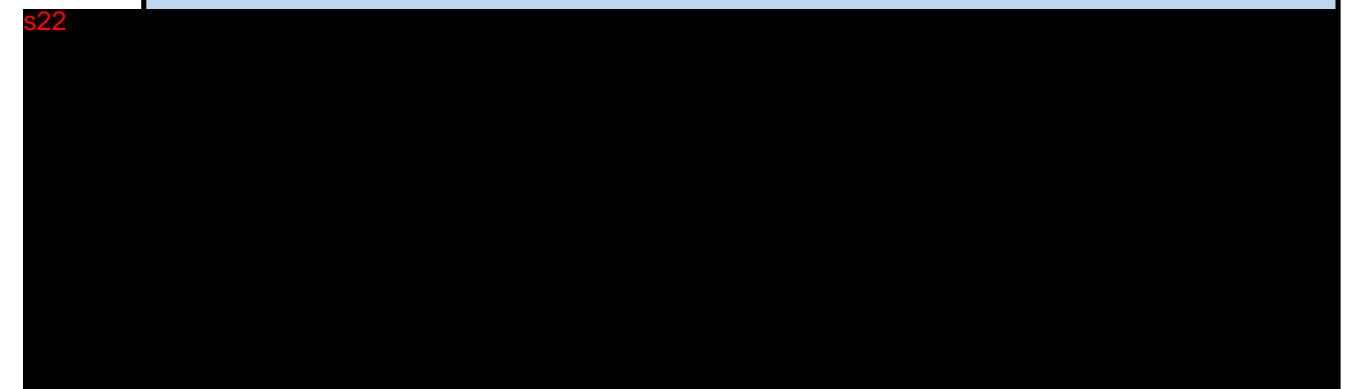
## Notes

Checked **s22** 28Apr2022

Data from Smear Analysis Table

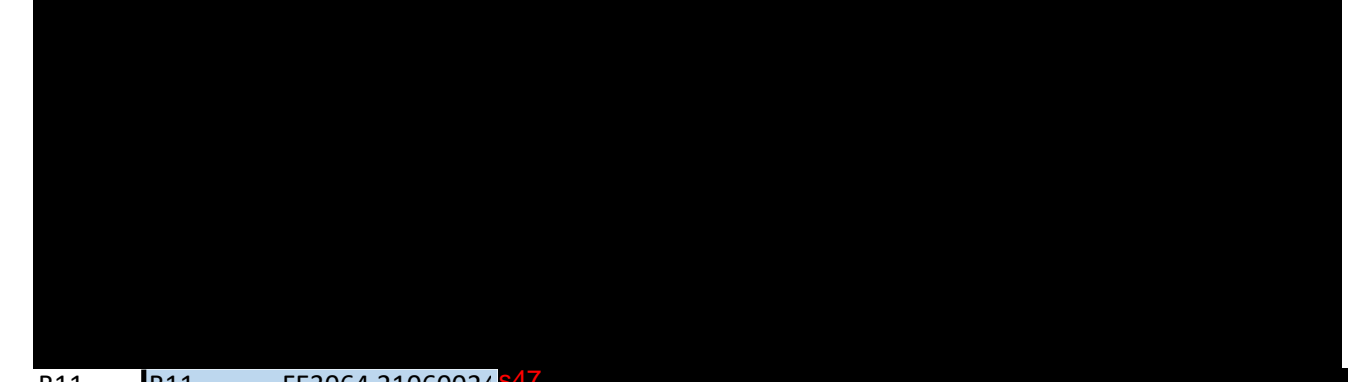
1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate
2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the two smear sets.
3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	A1	BLANK	3700 nt to 4800 nt	0.066	13.6	0.049	4208	6.7
A1	A1	BLANK	4800 nt to 13000 nt	0.0499	10.2	0.0199	7807	27.25
A2	A2	BLANK	3700 nt to 4800 nt	0.0473	9.5	0.0352	4185	8.05
A2	A2	BLANK	4800 nt to 13000 nt	0.0311	6.2	0.0134	7240	35.78
A3	A3	BLANK	3700 nt to 4800 nt	0.1603	9.9	0.1236	4046	5.65
A3	A3	BLANK	4800 nt to 13000 nt	0.0521	3.2	0.0205	7939	16.3
A4	A4	BLANK	3700 nt to 4800 nt	0.2309	3.4	0.1737	4147	7.47
A4	A4	BLANK	4800 nt to 13000 nt	0.0581	0.9	0.0274	6621	23.11
A5	A5	BLANK	3700 nt to 4800 nt	0.0587	4.2	0.0452	4048	6.66
A5	A5	BLANK	4800 nt to 13000 nt	0.0066	0.5	0.0025	8207	15.84



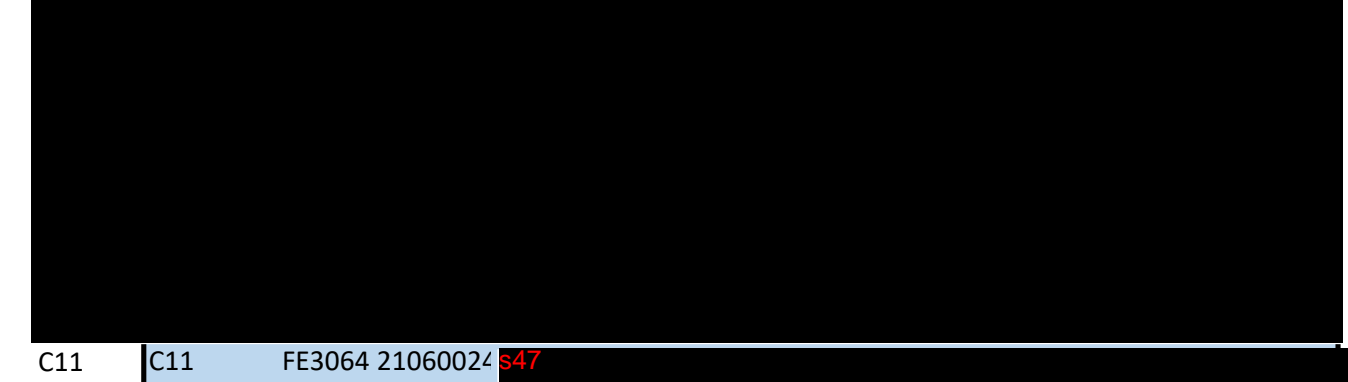
A11	A11	FE3064 21060024						
A11	A11	FE3064 21060024						

B1	B1	BLANK	3700 nt to 4800 nt	0.3549	3.2	0.2597	4264	6.99
B1	B1	BLANK	4800 nt to 13000 nt	0.0739	0.7	0.043	5358	23.81
B2	B2	BLANK	3700 nt to 4800 nt	NaN	NaN	NaN	3985	6.63
B2	B2	BLANK	4800 nt to 13000 nt	NaN	NaN	NaN	7862	31.08
B3	B3	BLANK	3700 nt to 4800 nt	0.0006	0	0.0004	4000	0
B3	B3	BLANK	4800 nt to 13000 nt	0.0057	0.1	0.0018	9849	0.68
B4	B4	BLANK	3700 nt to 4800 nt	0.0025	0.3	0.0017	4513	1.22
B4	B4	BLANK	4800 nt to 13000 nt	0.0113	1.4	0.0039	9072	22.95
B5	B5	BLANK	3700 nt to 4800 nt	0.063	1.2	0.0465	4222	6.49
B5	B5	BLANK	4800 nt to 13000 nt	0.0036	0.1	0.0011	9836	0



B11	B11	FE3064 21060024						
B11	B11	FE3064 21060024						

C1	C1	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C1	C1	BLANK	4800 nt to 13000 nt	0.0431	2.1	0.0136	9926	0.93
C2	C2	BLANK	3700 nt to 4800 nt	NaN	NaN	NaN	3968	7.03
C2	C2	BLANK	4800 nt to 13000 nt	NaN	NaN	NaN	7123	34
C3	C3	BLANK	3700 nt to 4800 nt	0.0281	1.4	0.0203	4324	3.84
C3	C3	BLANK	4800 nt to 13000 nt	0.0104	0.5	0.0033	9893	0.3
C4	C4	BLANK	3700 nt to 4800 nt	0.0072	1.1	0.0054	4176	7.09
C4	C4	BLANK	4800 nt to 13000 nt	0.0221	3.4	0.0075	9172	21.59
C5	C5	BLANK	3700 nt to 4800 nt	0.1021	2.2	0.0708	4500	5.82
C5	C5	BLANK	4800 nt to 13000 nt	0.0465	1	0.024	6057	28.08



C11	C11	FE3064 21060024						
C11	C11	FE3064 21060024						

D1	D1	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D1	D1	BLANK	4800 nt to 13000 nt	0.032	2.9	0.0101	9872	0.88
D2	D2	BLANK	3700 nt to 4800 nt	0.0393	1.2	0.0275	4455	6.82
D2	D2	BLANK	4800 nt to 13000 nt	0.0049	0.1	0.0031	4863	0.31
D3	D3	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D3	D3	BLANK	4800 nt to 13000 nt	0.0002	1.7	0.0001	8798	0.13
D4	D4	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D4	D4	BLANK	4800 nt to 13000 nt	0.0133	6.1	0.0043	9554	1.36
D5	D5	BLANK	3700 nt to 4800 nt	0.0042	1.8	0.0032	4157	5.08
D5	D5	BLANK	4800 nt to 13000 nt	0.0181	7.9	0.006	9480	4.91
D6	D6	BLANK	3700 nt to 4800 nt	0.0065	1.8	0.0051	3956	8.49
D6	D6	BLANK	4800 nt to 13000 nt	0.021	5.8	0.0067	9846	1.16
D7	D7	BLANK	3700 nt to 4800 nt	0.1132	13	0.0845	4179	8.29
D7	D7	BLANK	4800 nt to 13000 nt	0.0483	5.5	0.0226	6664	26.95
D8	D8	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D8	D8	BLANK	4800 nt to 13000 nt	0.0003	100	0.0001	9240	0
D9	D9	BLANK	3700 nt to 4800 nt	0.0509	49	0.04	3973	6.36
D9	D9	BLANK	4800 nt to 13000 nt	0.0049	4.7	0.0021	7392	20.73
D10	D10	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D10	D10	BLANK	4800 nt to 13000 nt	0.0096	100	0.0033	9107	1.63
D11	D11	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D11	D11	BLANK	4800 nt to 13000 nt	0.0081	100	0.003	8396	2.37
D12	D12	LADDER						

Written By §22  
 Authorised §22  
 Date issued 10/06/2021  
 Revision no. 0  
 LIMS number 33325

Date Validated 10/06/2021  
 Validation Due 10/06/2022  
 Validation Status Validation OVERDUE  
 Analyst §22  
 Assay Date 1/07/2021

Pass/Fail Parameters

minimum	cut off	maximum
<span style="color: red;">§47</span>		
result >>	<span style="color: red;">§47</span>	

										% INTEGRITY SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
1	A1	BLANK	3700 nt to 4800 nt	0.066	13.6	0.049	4208	6.7						
2	B1	BLANK	3700 nt to 4800 nt	0.3549	3.2	0.2597	4264	6.99	BLANK	5.60	7.11	126.97	FAIL	
3	C1	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	A2	BLANK	3700 nt to 4800 nt	0.0473	9.5	0.0352	4185	8.05						
2	B2	BLANK	3700 nt to 4800 nt	NaN	NaN	NaN	3985	6.63	BLANK	9.50	#DIV/0!	#DIV/0!	FAIL	
3	C2	BLANK	3700 nt to 4800 nt	NaN	NaN	NaN	3968	7.03						
1	A3	BLANK	3700 nt to 4800 nt	0.1603	9.9	0.1236	4046	5.65						
2	B3	BLANK	3700 nt to 4800 nt	0.0006	0	0.0004	4000	0	BLANK	3.77	5.36	142.24	FAIL	
3	C3	BLANK	3700 nt to 4800 nt	0.0281	1.4	0.0203	4324	3.84						
1	A4	BLANK	3700 nt to 4800 nt	0.2309	3.4	0.1737	4147	7.47						
2	B4	BLANK	3700 nt to 4800 nt	0.0025	0.3	0.0017	4513	1.22	BLANK	1.60	1.61	100.58	FAIL	
3	C4	BLANK	3700 nt to 4800 nt	0.0072	1.1	0.0054	4176	7.09						
1	A5	BLANK	3700 nt to 4800 nt	0.0587	4.2	0.0452	4048	6.66						
2	B5	BLANK	3700 nt to 4800 nt	0.063	1.2	0.0465	4222	6.49	BLANK	2.53	1.53	60.30	FAIL	
3	C5	BLANK	3700 nt to 4800 nt	0.1021	2.2	0.0708	4500	5.82						
§22														
1	A11	FE3064 2106002467							FE3064 2106002467				PASS	
2	B11	FE3064 2106002467												
3	C11	FE3064 2106002467												
§22														
1	D1	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	BLANK	0.40	0.69	173.21	FAIL	
2	D2	BLANK	3700 nt to 4800 nt	0.0393	1.2	0.0275	4455	6.82						
3	D3	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
1	D4	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN						
2	D5	BLANK	3700 nt to 4800 nt	0.0042	1.8	0.0032	4157	5.08	BLANK	1.20	1.04	86.60	FAIL	
3	D6	BLANK	3700 nt to 4800 nt	0.0065	1.8	0.0051	3956	8.49						
1	D7	BLANK	3700 nt to 4800 nt	0.1132	13	0.0845	4179	8.29						
2	D8	BLANK	3700 nt to 4800 nt	0	0	NaN	NaN	NaN	BLANK	20.67	25.38	122.82	FAIL	
3	D9	BLANK	3700 nt to 4800 nt	0.0509	49	0.04	3973	6.36						

										% LATE MIGRATING SPECIES SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
1	A1	BLANK	4800 nt to 13000 nt	0.0499	10.2	0.0199	7807	27.25						
2	B1	BLANK	4800 nt to 13000 nt	0.0739	0.7	0.043	5358	23.81	BLANK	4.33	5.13	118.35		
3	C1	BLANK	4800 nt to 13000 nt	0.0431	2.1	0.0136	9926	0.93						
1	A2	BLANK	4800 nt to 13000 nt	0.0311	6.2	0.0134	7240	35.78						
2	B2	BLANK	4800 nt to 13000 nt	NaN	NaN	NaN	7862	31.08	BLANK	6.20	#DIV/0!	#DIV/0!		
3	C2	BLANK	4800 nt to 13000 nt	NaN	NaN	NaN	7123	34						
1	A3	BLANK	4800 nt to 13000 nt	0.0521	3.2	0.0205	7939	16.3						
2	B3	BLANK	4800 nt to 13000 nt	0.0057	0.1	0.0018	9849	0.68	BLANK	1.27	1.69	133.12		
3	C3	BLANK	4800 nt to 13000 nt	0.0104	0.5	0.0033	9893	0.3						
1	A4	BLANK	4800 nt to 13000 nt	0.0581	0.9	0.0274	6621	23.11						
2	B4	BLANK	4800 nt to 13000 nt	0.0113	1.4	0.0039	9072	22.95	BLANK	1.90	1.32	69.63		
3	C4	BLANK	4800 nt to 13000 nt	0.0221	3.4	0.0075	9172	21.59						
1	A5	BLANK	4800 nt to 13000 nt	0.0066	0.5	0.0025	8207	15.84						
2	B5	BLANK	4800 nt to 13000 nt	0.0036	0.1	0.0011	9836	0	BLANK	0.53	0.45	84.55		
3	C5	BLANK	4800 nt to 13000 nt	0.0465	1	0.024	6057	28.08						
§22														
cessive baseline shifting and will be omitted. Average for this Sample will be calculated using wells B9 and C9 or														

b22										b22				
1	A11	FE3064 2106002467								b47				
2	B11	FE3064 2106002467								FE3064 2106002467				
3	C11	FE3064 2106002467												
b22														
1	D1	BLANK	4800 nt to 13000 nt	0.032	2.9	0.0101	9872	0.88						
2	D2	BLANK	4800 nt to 13000 nt	0.0049	0.1	0.0031	4863	0.31	BLANK	1.57	1.40	89.67		
3	D3	BLANK	4800 nt to 13000 nt	0.0002	1.7	0.0001	8798	0.13						
1	D4	BLANK	4800 nt to 13000 nt	0.0133	6.1	0.0043	9554	1.36						
2	D5	BLANK	4800 nt to 13000 nt	0.0181	7.9	0.006	9480	4.91	BLANK	6.60	1.14	17.21		
3	D6	BLANK	4800 nt to 13000 nt	0.021	5.8	0.0067	9846	1.16						
1	D7	BLANK	4800 nt to 13000 nt	0.0483	5.5	0.0226	6664	26.95						
2	D8	BLANK	4800 nt to 13000 nt	0.0003	100	0.0001	9240	0	BLANK	36.73	54.79	149.16		
3	D9	BLANK	4800 nt to 13000 nt	0.0049	4.7	0.0021	7392	20.73						





### VALIDATION DATA

REPLICATE	Well	Sample ID	Range	ng/uL	% Total
1	A1	sample1-rep1	3500 nt to 5389 nt	5	10
2	B1	sample1-rep2	3500 nt to 5389 nt	5.5	11
3	C1	sample1-rep3	3500 nt to 5389 nt	6	12
1	A2	sample2-rep1	3500 nt to 5389 nt	10	20
2	B2	sample2-rep2	3500 nt to 5389 nt	10.5	21
3	C2	sample2-rep3	3500 nt to 5389 nt	11	22
1	A3	sample3-rep1	3500 nt to 5389 nt	15	30
2	B3	sample3-rep2	3500 nt to 5389 nt	15.5	31
3	C3	sample3-rep3	3500 nt to 5389 nt	16	32
1	A4	sample4-rep1	3500 nt to 5389 nt	20	40
2	B4	sample4-rep2	3500 nt to 5389 nt	20.5	41
3	C4	sample4-rep3	3500 nt to 5389 nt	21	42
1	A5	sample5-rep1	3500 nt to 5389 nt	25	50
2	B5	sample5-rep2	3500 nt to 5389 nt	25.5	51
3	C5	sample5-rep3	3500 nt to 5389 nt	26	52
1	A6	sample6-rep1	3500 nt to 5389 nt	30	60
2	B6	sample6-rep2	3500 nt to 5389 nt	30.5	61
3	C6	sample6-rep3	3500 nt to 5389 nt	31	62
1	A7	sample7-rep1	3500 nt to 5389 nt	35	70
2	B7	sample7-rep2	3500 nt to 5389 nt	35.5	71
3	C7	sample7-rep3	3500 nt to 5389 nt	36	72

REPLICATE	Well	Sample ID	Range	ng/uL	% Total
1	A1	sample1-rep1	5389 nt to 13000 nt	0.5	1
2	B1	sample1-rep2	5389 nt to 13000 nt	1.05	2
3	C1	sample1-rep3	5389 nt to 13000 nt	1.6	3
1	A2	sample2-rep1	5389 nt to 13000 nt	1	2
2	B2	sample2-rep2	5389 nt to 13000 nt	1.55	3
3	C2	sample2-rep3	5389 nt to 13000 nt	2.1	4
1	A3	sample3-rep1	5389 nt to 13000 nt	1.5	3
2	B3	sample3-rep2	5389 nt to 13000 nt	2.05	4
3	C3	sample3-rep3	5389 nt to 13000 nt	2.6	5
1	A4	sample4-rep1	5389 nt to 13000 nt	2	4
2	B4	sample4-rep2	5389 nt to 13000 nt	2.55	5
3	C4	sample4-rep3	5389 nt to 13000 nt	3.1	6
1	A5	sample5-rep1	5389 nt to 13000 nt	2.5	5
2	B5	sample5-rep2	5389 nt to 13000 nt	3.05	6
3	C5	sample5-rep3	5389 nt to 13000 nt	3.6	7
1	A6	sample6-rep1	5389 nt to 13000 nt	3	6
2	B6	sample6-rep2	5389 nt to 13000 nt	3.55	7
3	C6	sample6-rep3	5389 nt to 13000 nt	4.1	8
1	A7	sample7-rep1	5389 nt to 13000 nt	3.5	7
2	B7	sample7-rep2	5389 nt to 13000 nt	4.05	8



3	C7	sample7-rep3	5389 nt to 13000 nt	4.6	9
---	----	--------------	---------------------	-----	---

nmole/L	Avg. Size	%CV
12.5	4079	2
13.5	4091	2.2
14.5	4089	2.4
22.5	4053	4
23.5	4061	4.2
24.5	4065	4.4
32.5	4045	6
33.5	4033	6.2
34.5	4037	6.4
42.5	4089	8
43.5	4069	8.2
44.5	4061	8.4
52.5	4061	10
53.5	4067	10.2
54.5	4070	10.4
62.5	4009	12
63.5	3998	12.2
64.5	4097	12.4
72.5	4071	14
73.5	4049	14.2
74.5	4060	14.4
nmole/L	Avg. Size	%CV
3.5	6774	0.2
4.6	5534	0.42
5.7	5684	0.64
4.5	6916	0.4
5.6	4079	0.62
6.7	5530	0.84
5.5	6870	0.6
6.6	6807	0.82
7.7	6551	1.04
6.5	7320	0.8
7.6	7000	1.02
8.7	6970	1.24
7.5	7135	1
8.6	7094	1.22
9.7	6740	1.44
8.5	4079	1.2
9.6	5436	1.42
10.7	8653	1.64
9.5	7717	1.4
10.6	7570	1.62

REPLICATE	Well	Sample ID
1	A1	sample1-rep1
2	B1	sample1-rep2
3	C1	sample1-rep3
1	A2	sample2-rep1
2	B2	sample2-rep2
3	C2	sample2-rep3
1	A3	sample3-rep1
2	B3	sample3-rep2
3	C3	sample3-rep3
1	A4	sample4-rep1
2	B4	sample4-rep2
3	C4	sample4-rep3
1	A5	sample5-rep1
2	B5	sample5-rep2
3	C5	sample5-rep3
1	A6	sample6-rep1
2	B6	sample6-rep2
3	C6	sample6-rep3
1	A7	sample7-rep1
2	B7	sample7-rep2
3	C7	sample7-rep3
REPLICATE	Well	Sample ID
1	A1	sample1-rep1
2	B1	sample1-rep2
3	C1	sample1-rep3
1	A2	sample2-rep1
2	B2	sample2-rep2
3	C2	sample2-rep3
1	A3	sample3-rep1
2	B3	sample3-rep2
3	C3	sample3-rep3
1	A4	sample4-rep1
2	B4	sample4-rep2
3	C4	sample4-rep3
1	A5	sample5-rep1
2	B5	sample5-rep2
3	C5	sample5-rep3
1	A6	sample6-rep1
2	B6	sample6-rep2
3	C6	sample6-rep3
1	A7	sample7-rep1
2	B7	sample7-rep2

11.7 8404 1.84

3 C7 sample7-rep3

## RESULTS FOR VALIDATION

Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
3500 nt to 5389 nt	5	10	12.5	4079	2
3500 nt to 5389 nt	5.5	11	13.5	4091	2.2
3500 nt to 5389 nt	6	12	14.5	4089	2.4
3500 nt to 5389 nt	10	20	22.5	4053	4
3500 nt to 5389 nt	10.5	21	23.5	4061	4.2
3500 nt to 5389 nt	11	22	24.5	4065	4.4
3500 nt to 5389 nt	15	30	32.5	4045	6
3500 nt to 5389 nt	15.5	31	33.5	4033	6.2
3500 nt to 5389 nt	16	32	34.5	4037	6.4
3500 nt to 5389 nt	20	40	42.5	4089	8
3500 nt to 5389 nt	20.5	41	43.5	4069	8.2
3500 nt to 5389 nt	21	42	44.5	4061	8.4
3500 nt to 5389 nt	25	50	52.5	4061	10
3500 nt to 5389 nt	25.5	51	53.5	4067	10.2
3500 nt to 5389 nt	26	52	54.5	4070	10.4
3500 nt to 5389 nt	30	60	62.5	4009	12
3500 nt to 5389 nt	30.5	61	63.5	3998	12.2
3500 nt to 5389 nt	31	62	64.5	4097	12.4
3500 nt to 5389 nt	35	70	72.5	4071	14
3500 nt to 5389 nt	35.5	71	73.5	4049	14.2
3500 nt to 5389 nt	36	72	74.5	4060	14.4

Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
5389 nt to 13000 nt	0.5	1	3.5	6774	0.2
5389 nt to 13000 nt	1.05	2	4.6	5534	0.42
5389 nt to 13000 nt	1.6	3	5.7	5684	0.64
5389 nt to 13000 nt	1	2	4.5	6916	0.4
5389 nt to 13000 nt	1.55	3	5.6	4079	0.62
5389 nt to 13000 nt	2.1	4	6.7	5530	0.84
5389 nt to 13000 nt	1.5	3	5.5	6870	0.6
5389 nt to 13000 nt	2.05	4	6.6	6807	0.82
5389 nt to 13000 nt	2.6	5	7.7	6551	1.04
5389 nt to 13000 nt	2	4	6.5	7320	0.8
5389 nt to 13000 nt	2.55	5	7.6	7000	1.02
5389 nt to 13000 nt	3.1	6	8.7	6970	1.24
5389 nt to 13000 nt	2.5	5	7.5	7135	1
5389 nt to 13000 nt	3.05	6	8.6	7094	1.22
5389 nt to 13000 nt	3.6	7	9.7	6740	1.44
5389 nt to 13000 nt	3	6	8.5	4079	1.2
5389 nt to 13000 nt	3.55	7	9.6	5436	1.42
5389 nt to 13000 nt	4.1	8	10.7	8653	1.64
5389 nt to 13000 nt	3.5	7	9.5	7717	1.4
5389 nt to 13000 nt	4.05	8	10.6	7570	1.62

5389 nt to 13000 nt	4.6	9	11.7	8404	1.84
---------------------	-----	---	------	------	------

## DATA

## % INTEGRITY SUMMARY

Sample ID	Average	stdev	%CV
sample1-rep1	11.0	1.0	9.1
sample2-rep1	21.0	1.0	4.8
sample3-rep1	31.0	1.0	3.2
sample4-rep1	41.0	1.0	2.4
sample5-rep1	51.0	1.0	2.0
sample6-rep1	61.0	1.0	1.6
sample7-rep1	71.0	1.0	1.4

Sample ID	Average	stdev	%CV
sample1-rep1	2.0	1.0	50.0
sample2-rep1	3.0	1.0	33.3
sample3-rep1	4.0	1.0	25.0
sample4-rep1	5.0	1.0	20.0
sample5-rep1	6.0	1.0	16.7
sample6-rep1	7.0	1.0	14.3
sample7-rep1	8.0	1.0	12.5



FAIL

FAIL

FAIL

FAIL

FAIL

PASS

PASS





## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2021 07 01\14-35-27\2021 07 01 14H 35M.raw  
**Created:** Thursday, July 1, 2021 3:00:51 PM  
**Number of capillaries:** 24  
**Array serial number:** 022621-27SFS  
**Effect length:** 33 cm  
**Array usage count:** 9  
**Instrument type:** 5300 Fragment Analyzer  
**Instrument controller software version:** 3.1.0.12  
**Device serial number:** MY2105AB19

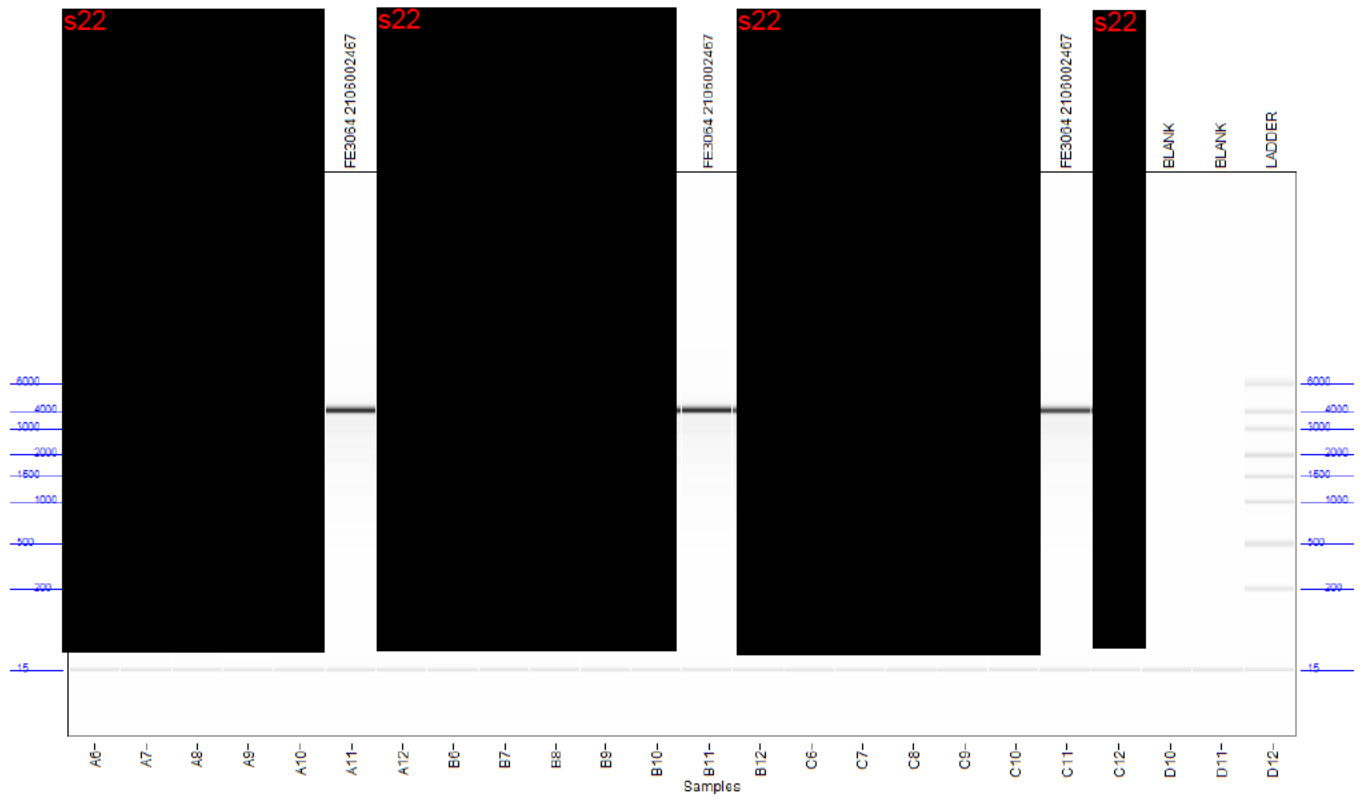
### Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds  
**Gel prime:** No  
**Full conditioning:** Yes  
**Gel prime to buffer:** Yes  
**Gel selection:** Gel 2  
**Perform prerun:** 8.0 kV, 30 sec.  
**Rinse:** No  
**Marker 1:** No  
**Rinse:** Tray: 3, Row: A, Dip count: 2  
**Sample injection:** 5.0 kV, 6 sec.  
**Separation:** 8.0 kV, 60.0 min.  
**Tray name:** Tray-1

**Analysis mode:** RNA (Eukaryotic)

### Notes

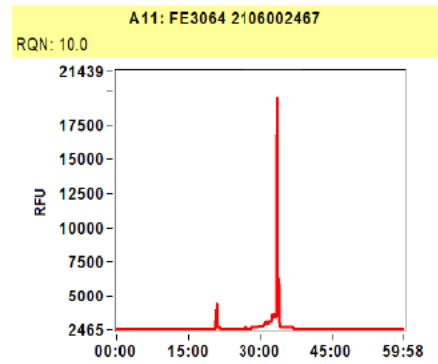
### Gel Image



Filename and data path: C:\Agilent Technologies\Data\2021 07 01\14-35-27\2021 07 01 14H 35M.raw

s22

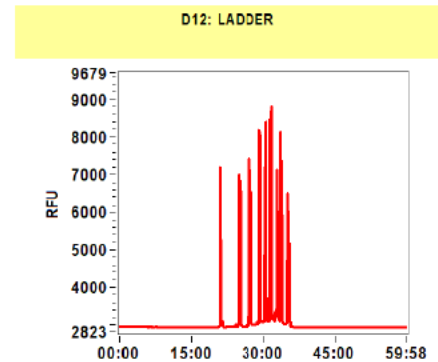
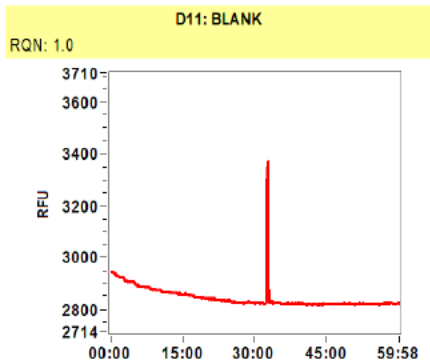
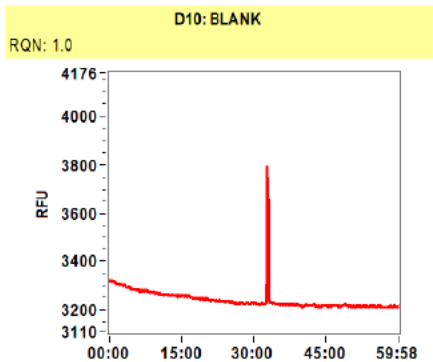
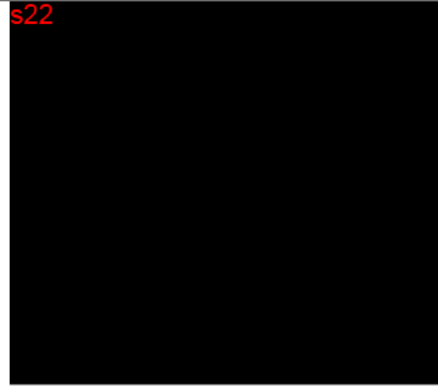
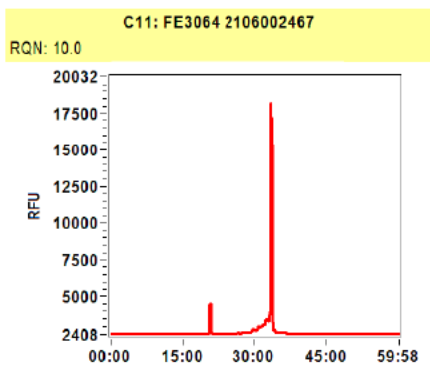
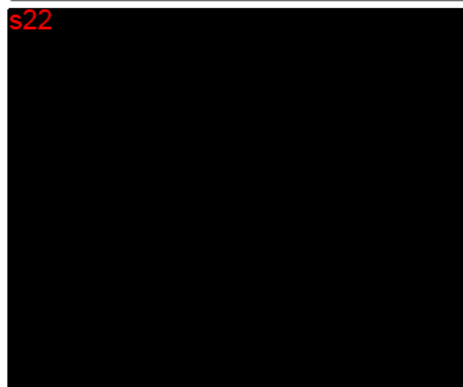
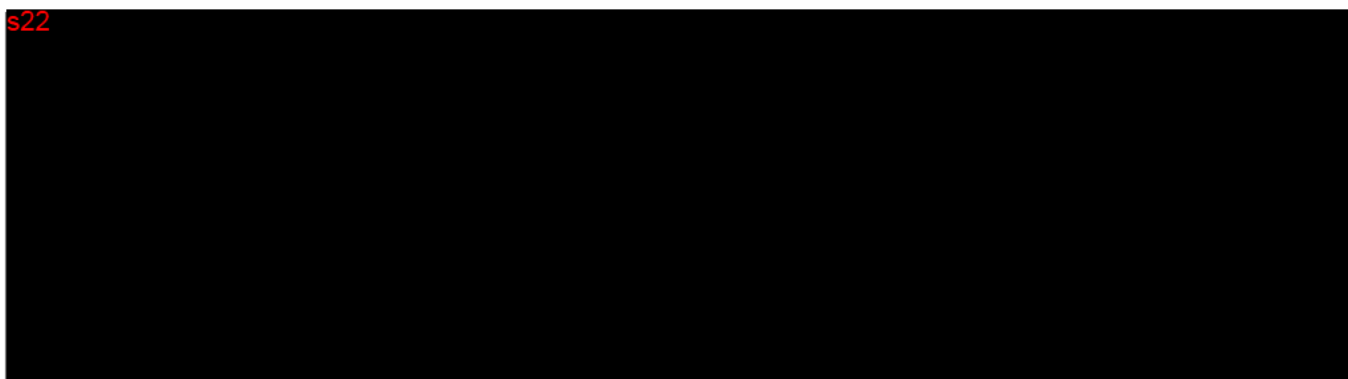
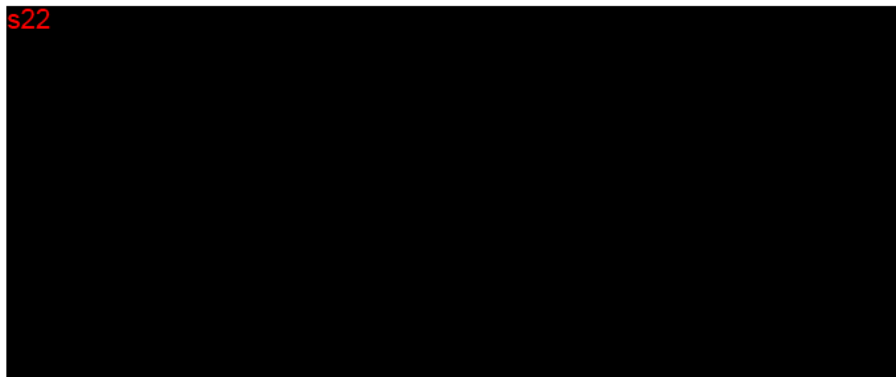
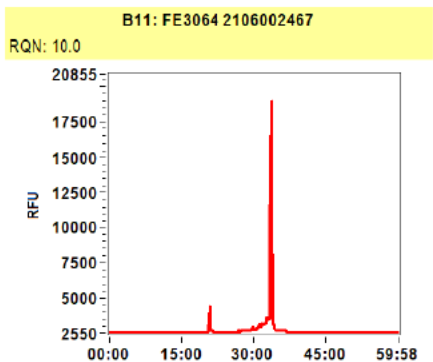
s22



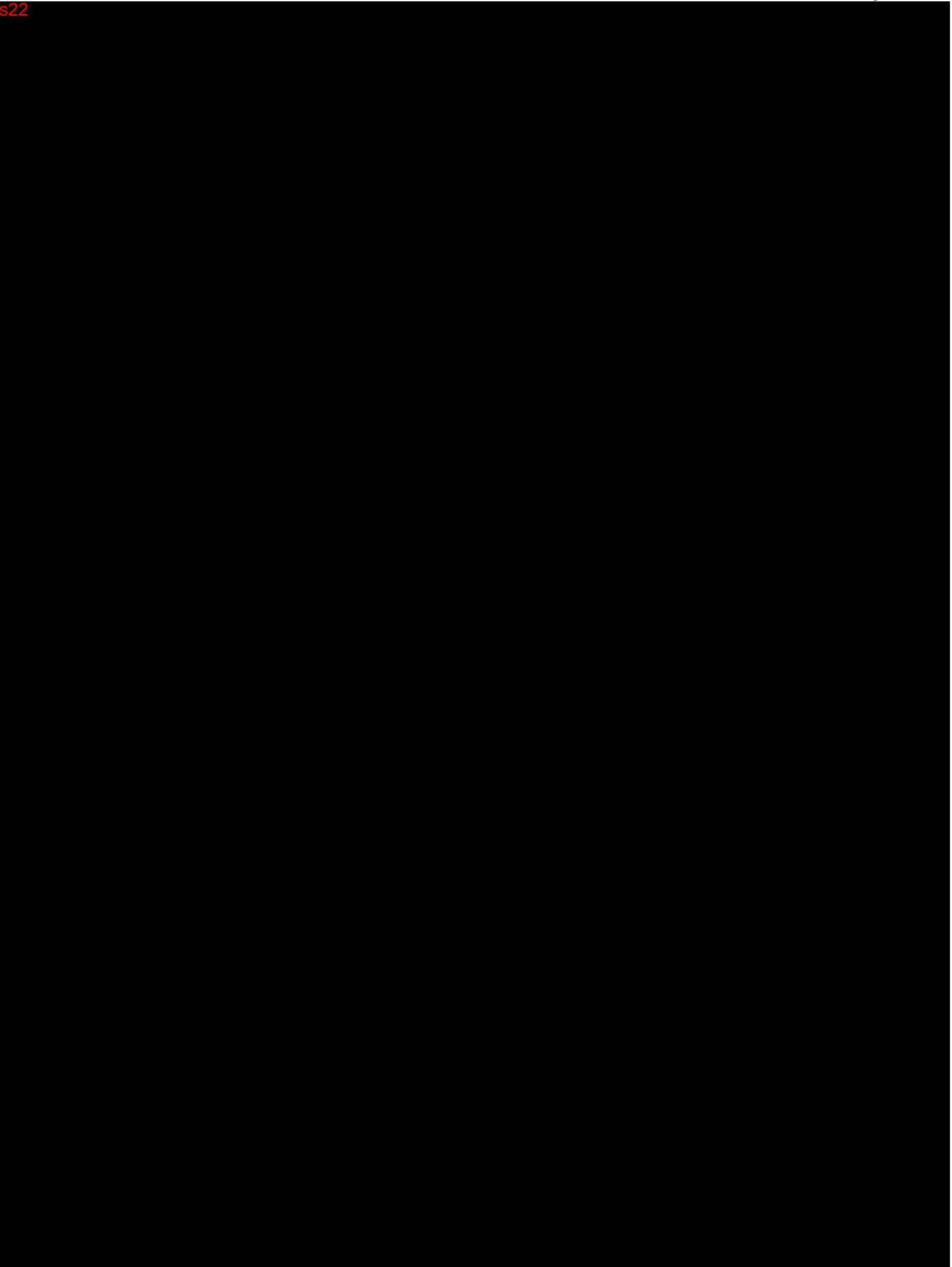
s22

s22

Filename and data path: C:\Agilent Technologies\Data\2021 07 01\14-35-27\2021 07 01 14H 35M.raw



s22



s22



s22





s22



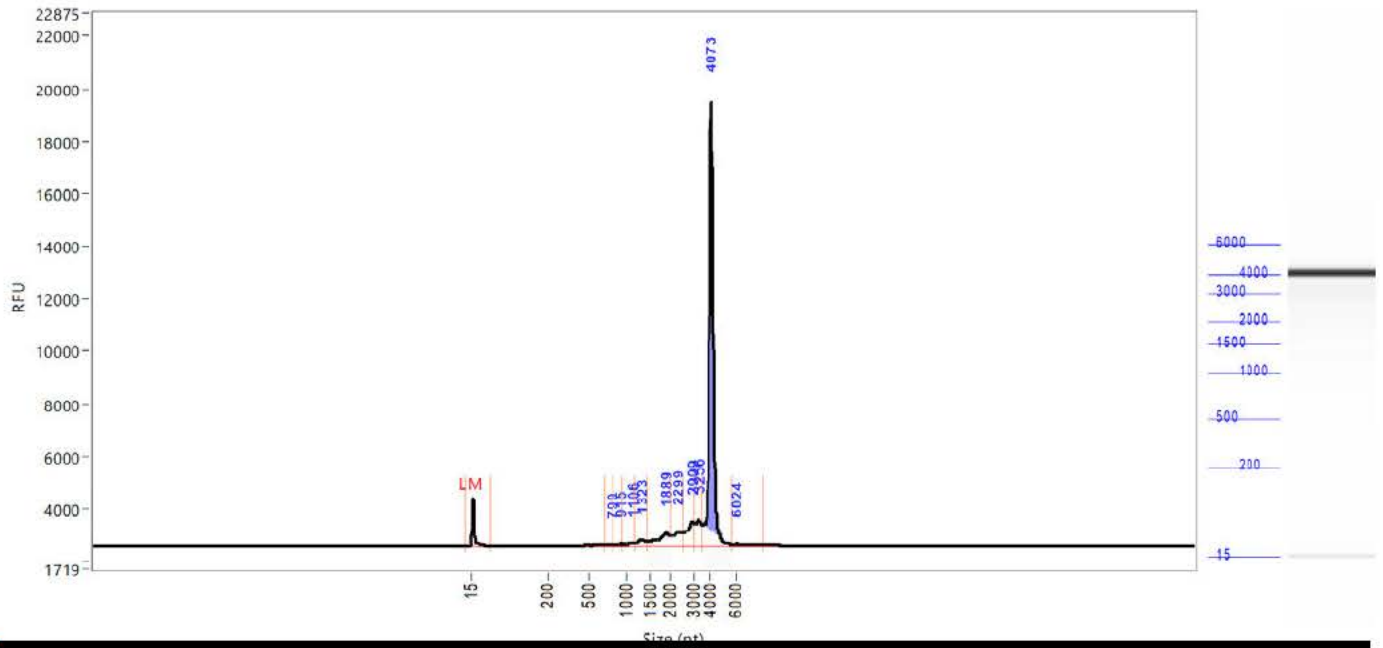
S22



s22



s22



s47

s22



s22



s22



s22





s22

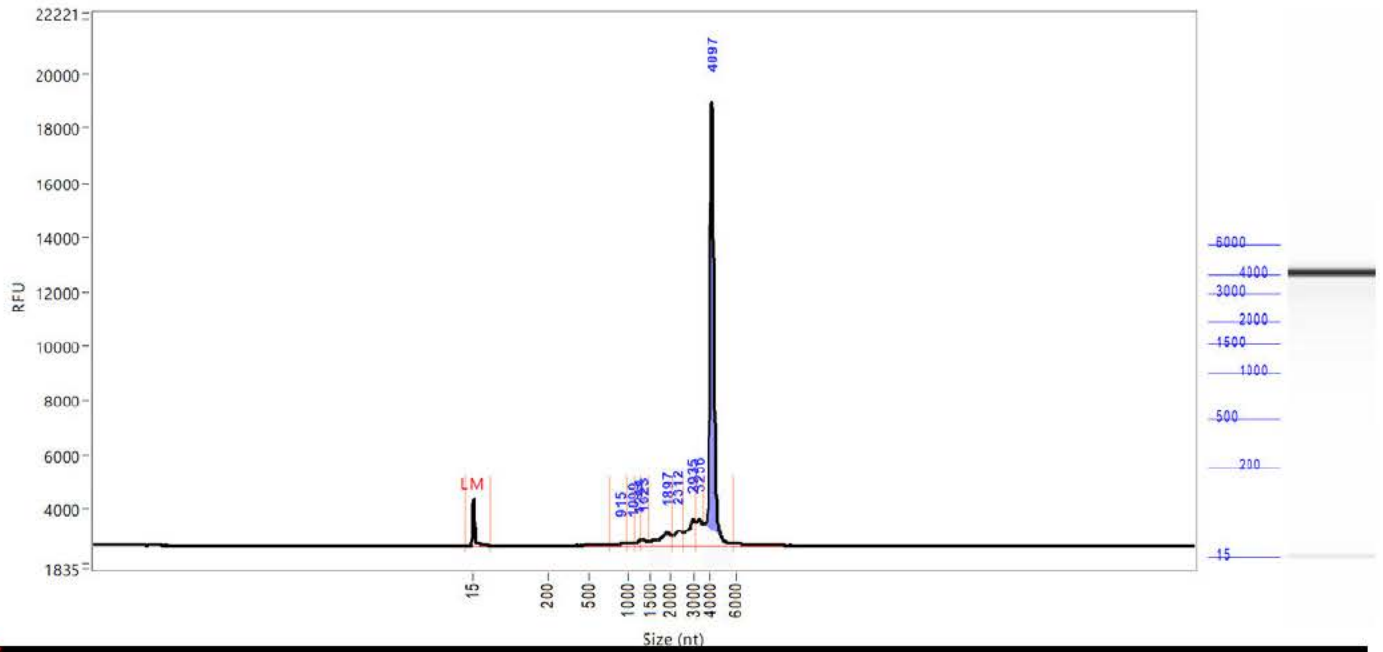


s22



Sample: FF3064\_2106002467

s22



s47

s22



s22



S22



s22



s22



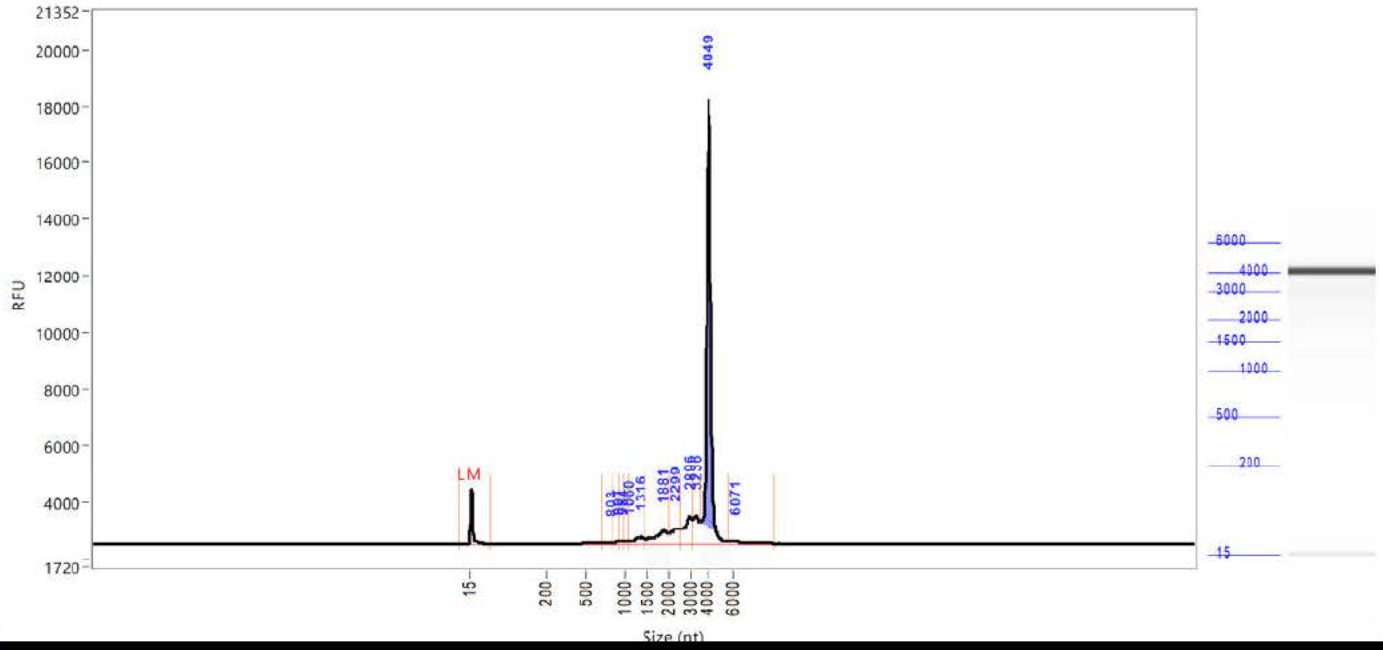


s22



Sample: FE3064 2106002467

s22

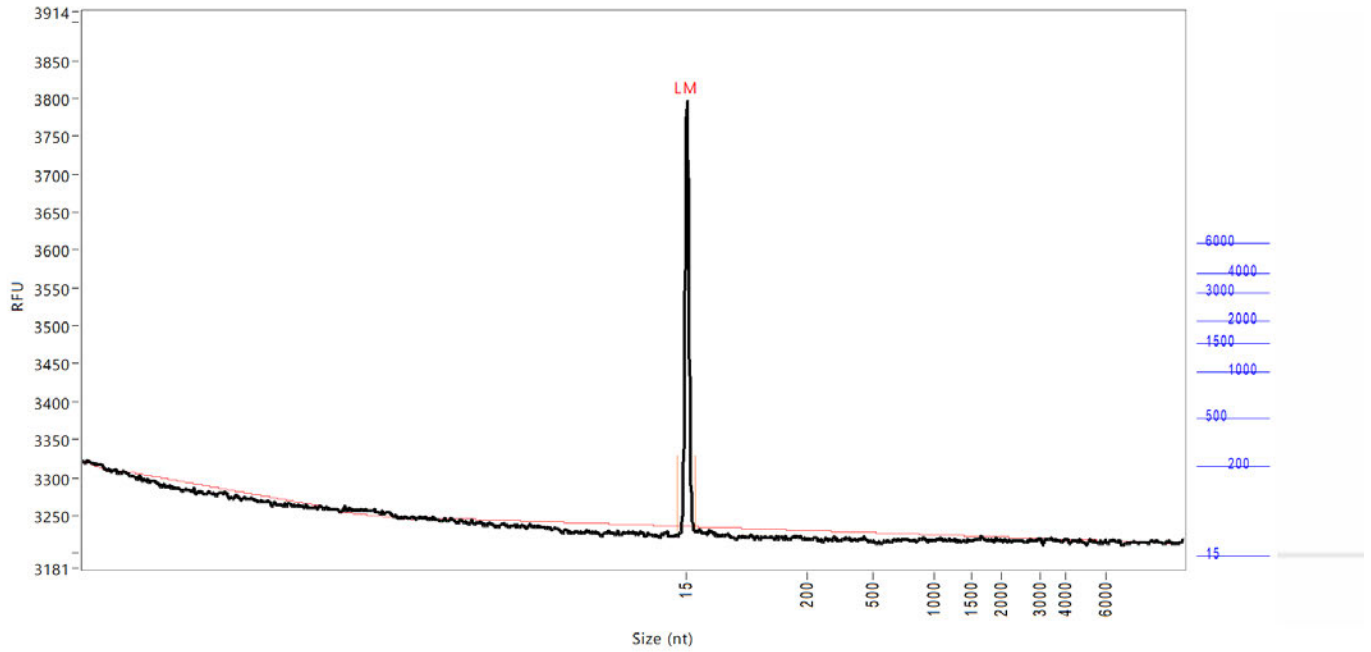


s47

S22



**Sample:** BLANK  
**Well location:** D10  
**Created:** Thursday, July 1, 2021 3:00:51 PM

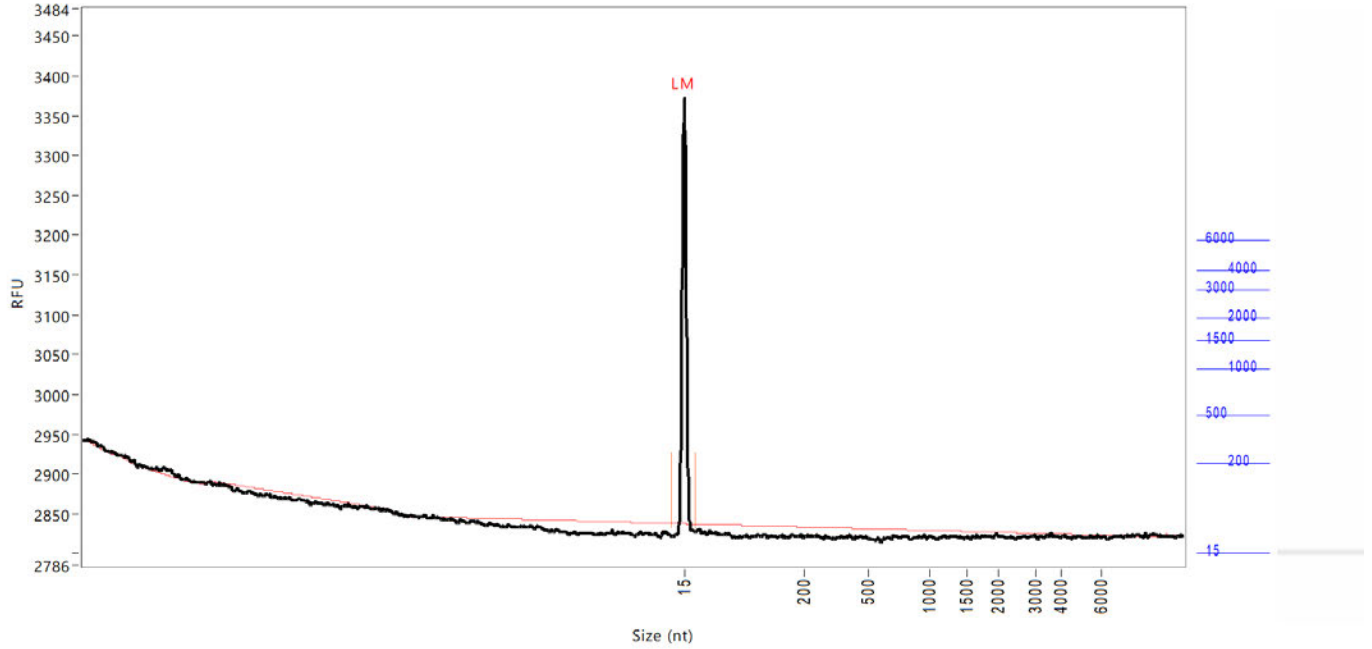


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.8168	0	27	559
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0096	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/uL	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0096 ng/uL	100.0 %Total	0.0033 nmole/L	9107 Avg. Size (nt)	1.63 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** BLANK  
**Well location:** D11  
**Created:** Thursday, July 1, 2021 3:00:51 PM

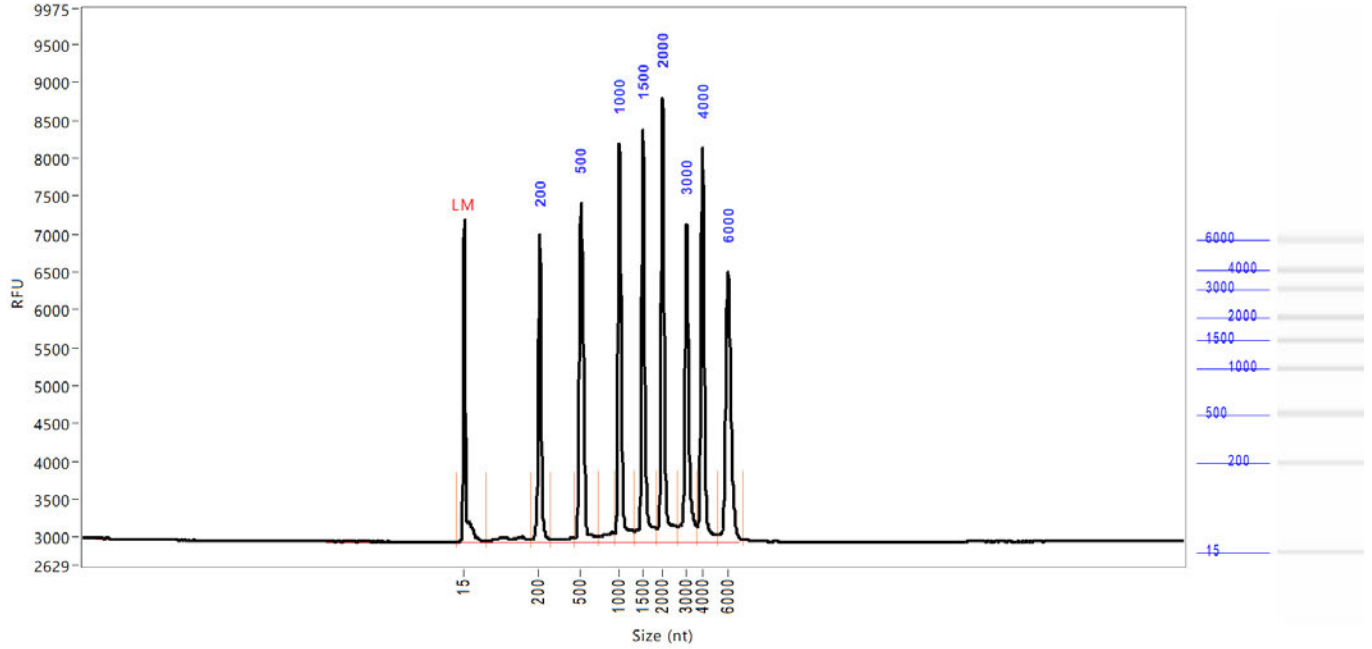


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.8168	0	32	533
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0081	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/uL	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0081 ng/uL	100.0 %Total	0.0030 nmole/L	8396 Avg. Size (nt)	2.37 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** LADDER  
**Well location:** D12  
**Created:** Thursday, July 1, 2021 3:00:51 PM



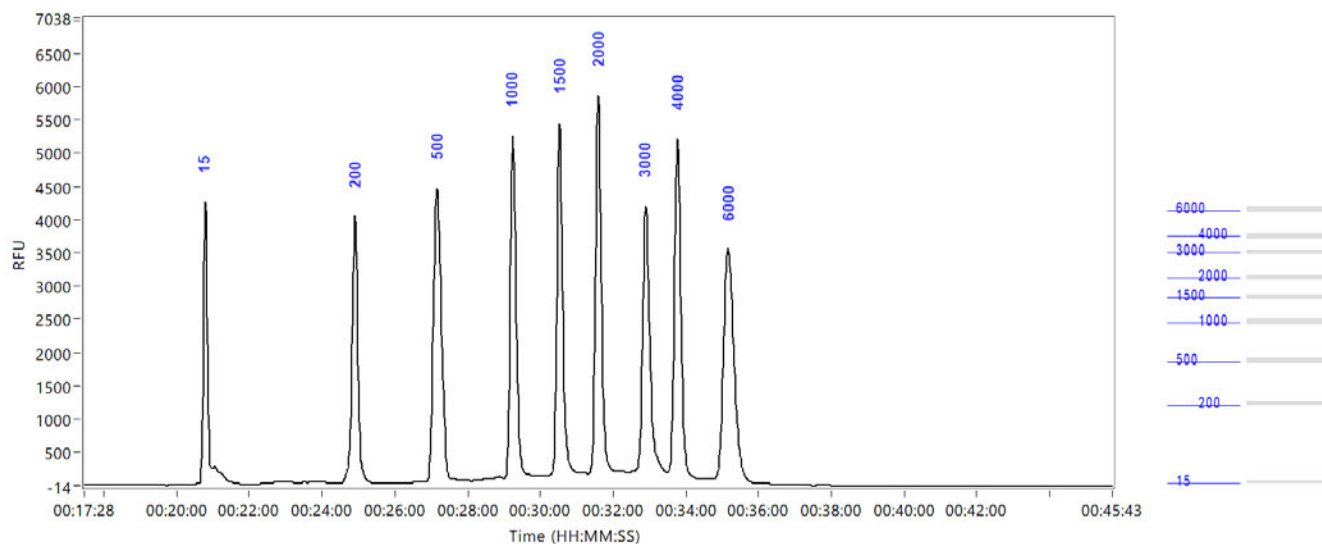
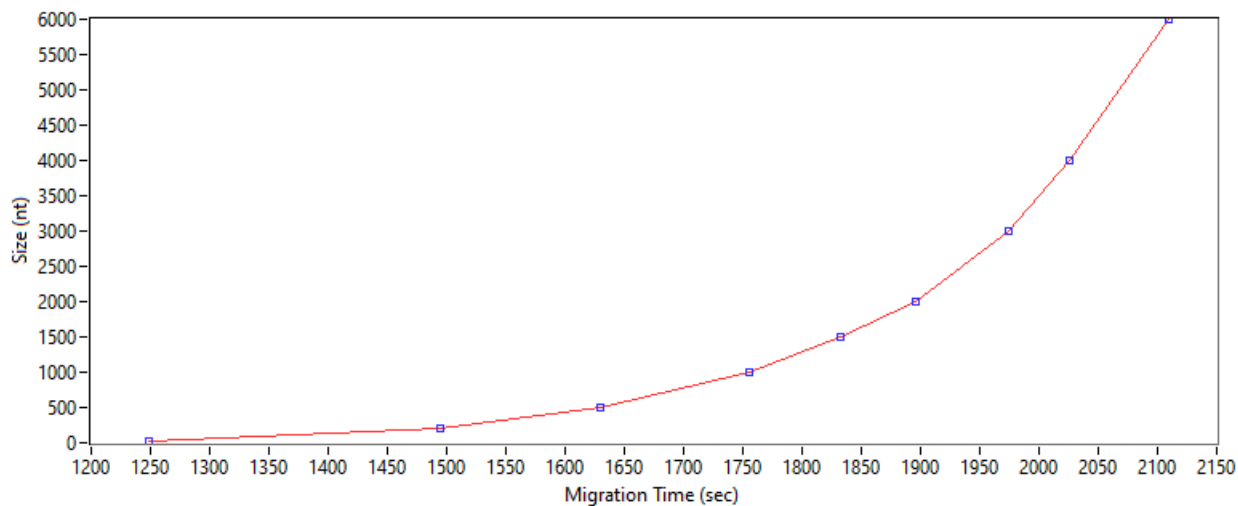
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.8168	0	69	4260
2	200	10.0139	180	277	4051
3	500	14.2209	453	722	4469
4	1000	11.9784	940	1310	5253
5	1500	12.1813	1310	1865	5448
6	2000	12.9166	1865	2624	5863
7	3000	10.8039	2624	3687	4194
8	4000	11.3358	3687	5253	5202
9	6000	11.2335	5253	7167	3559

TIC: 94.6842 ng/uL  
 TIM: 349.3954 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: Y    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt): 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** LADDER  
**Well location:** D12  
**Created:** Thursday, July 1, 2021 3:00:51 PM  
**Fit type:** Point to point

Calibration curve





<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Appendix 1 - Fragment Analyzer Worksheet - Pfizer COMIRNATY	

### Worksheet for Fragment Analyzer - RNA Integrity

Test Details			
<b>SOP QPulse #</b>	TRIM - D20-3875075	<b>Analysist</b>	s22
<b>TRIM link to data files</b>	D21-2799223, D21-2799226	<b>Test Date</b>	1/07/2021

Pipettes & Equipment	
Name	LIMS#
30-300 µL 12 channel pipette	-
p10 pipette	33087
p50 pipette	-
p100 pipette	32792
p200 pipette	5649
Thermomixer	32660
Thermocycler	32865
-	-

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	-
Standard Sensitivity (SS) RNA kit Part 1 stored at 2-8°C	DNF-471-0500	6591350	8/12/2021
<i>Extra Blank solution</i>	<i>DNF-300-0008</i>	-	-
Standard Sensitivity (SS) RNA kit Part 2 stored at -20°C (Diluent Marker & Intercalating dye)	-	6595832	8/03/2022
<i>Extra Diluent marker</i>	<i>DNF-369-0004</i>	-	-
Standard Sensitivity (SS) RNA kit Part 3 stored at -70°C (RNA Ladder)	DNF-382-U020	6595344	8/03/2022
Capillary conditioning solution	DNF-475-0100	6578326	9/12/2021
DEPC water	Am9961	2004017	-
20% Triton-X100 / 30% Ethanol solution	In house	MC1JUN21-01	1/09/2021
Enter text.	Enter text.	Enter text.	Enter a date.
Enter text.	Enter text.	Enter text.	Enter a date.
Enter text.	Enter text.	Enter text.	Enter a date.



Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT ( <b>Drawer B</b> )	1/07/2021	2/07/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT ( <b>Drawer M</b> )	1/07/2021	2/07/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT ( <b>Drawer 3</b> )	18/06/2021	2/07/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	1/07/2021	15/07/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	1/07/2021	3/07/2021  48 hours
<b>Empty waste tray and waste bottle</b> <b>Reagents can be scaled up if required – this table provides the minimum for a single run.</b> <b>Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</b> <b>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</b>  <b>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</b>			

#### 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	BF-25	BF-25	BF-25	BF-25	BF-25	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	BF-25	BF-25	BF-25	BF-25	BF-25	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	BF-25	BF-25	BF-25	BF-25	BF-25	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	L

**S1-6** = Samples in triplicate (a, b or c),

**RM** = Reference material

**BF-25** = Blank solution provided in kit,

**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test. Any samples not included in the test must be crossed off the plate layout, and results table below

System Suitability Criteria – RNA Ladder			
Plate location (wells)	D12		
Parameter	Limits	Results	Comments
RNA ladder profile	Visually comparable to figure 4 of SOP	ok	PASS
All peaks present	15 200 500 1000 1500 2000 3000 4000 6000 nt	ok	PASS
Peak heights	<60000 RFU	ok	PASS
Assay Acceptance Criteria – Reference Material			
Plate location (wells)	A12 B12 C12		
LIMS #	2103001141		
BATCH #	EE8493		
EXPIRY	5/08/2021		
Parameter	Limits	Results	Comments
Profile	Visually comparable to DP electropherogram in SOP	Ok/ok/ok	PASS
Migration time	Approximately comparable to profile in SOP	4073/4073/4073	PASS
Lower marker present	LM peak	Ok/ok/ok	PASS
Peak heights	5000-600000 for 2/3 replicates	14497/12578/12384	PASS
No negative peaks or baseline shifts	No significant peaks/shifts	Ok/ok/ok	PASS
Reference Material Dilutions / Calculation / Notes			
thaw date: 01/07/21 270ng/uL = 20 uL of 530 ng/uL master stock + 19 uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution  RM – 57.93% integrity, 0.67 stdev, 1.15 %RSD			

Sample 1 Details	
Plate location (wells)	A11 B11 C11
LIMS #	2106002467
BATCH #	FE3064
EXPIRY	31/10/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4073/4097/4049	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	16917/16293/15693	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s47			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 2 Details	
Plate location (wells)	A10 B10 C10
LIMS #	2106002408
BATCH #	s22
EXPIRY	31/10/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4097/4073/4073	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	13612/13362/13588	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 3 Details	
Plate location (wells)	A9 B9 C9
LIMS #	2103001151
BATCH #	s22
EXPIRY	31/07/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4073/4025	PASS
Lower marker	LM must be present	Ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	16160/17997	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
<p>270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water                      90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>one of the replicates was excluded due to excessive baseline shifting. Averages are calculated from two replicates only.</p>

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 4 Details	
Plate location (wells)	A8 B8 C8
LIMS #	2103001420
BATCH #	s22
EXPIRY	31/07/2021

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4073/4097/4025	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	18710/16081/18762	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 5 Details	
Plate location (wells)	A7 B7 C7
LIMS #	2102000955
BATCH #	s22
EXPIRY	30/06/2021

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4073/4049/4025	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	18113/17594/18663	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22

Sample 6 Details	
Plate location (wells)	A6 B6 C6
LIMS #	2102000687
BATCH #	s22
EXPIRY	30/06/2021

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4073/4049/4001	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	15899/16118/17333	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	s47	s22			PASS
% Late Migrating Species					

Sample Dilutions / Calculation / Notes
270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution

Sample Results	
PASS	
Analysist	s22
Checked by	s22



**Notes**

Enter text.



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Operations	
Procedure	Bacterial Endotoxin Testing - Appendix 2K - Kinetic LAL Routine Assay Worksheet
Written	s22 and s22
Authorised	s22
Date issued	16 August 2017
Revision #	1

### Appendix 2K - ROUTINE ASSAY WORKSHEET

Assay ID: 29 JUN 21/i Operator: s22  
 (format DDMMYY/1)

**Limulus Amoebocyte Lysate (LAL)** - record details on Printed copy of Appendix 3

LAL Lot Number: WLO29JTCAW LAL Expiry: 21 OCT 2022

Date Reconstituted: 29 JUN 21 & 23 JUN 21 LRW lot: 0000830072  
 (USE BY 7 JUL 21)

Ensure sensitivity of LAL batch has been confirmed. Confirmation date: 01 JUN 2021

**Control Standard Endotoxin (CSE)** - record details on printed copy of Appendix 4

CSE Lot Number: 0000820346 CSE Expiry: 01 JUL 2023

Date Reconstituted: 29 JUN 2021 to 50 EU/ml with LRW: 0000830072

Use by Date: 27 JULY 2021 Use by date is 4 weeks (ie. 28 days) from reconstitution when stored at 2-8°C

**LAL Reagent Water (LRW)**

LRW Lot Number: 0000830072 LRW Expiry: 01 MAR 2022

How many samples were linked to this assay ? 4

**This Appendix is used for recording the assay details and results and only gives the method in point form. See the full SOP (Appendix 8K) for the detailed method.**

To avoid endotoxin contamination, use careful technique and **pyrogen free** equipment.

Record Details	D17-686127 Bacterial Endotoxin Testing - Appendix 2K - Kinetic LAL Routine Assay Worksheet.DOCX		
Last Editor	s22	Edit Date	22/08/2017 11:23 AM
Print Date	29/06/2021 12:17 PM		Page 1 of 4

Preparation of Software

- Print and fill out the appropriate paperwork from the Quality Management System.
- Retrieve the required kit reagents from cold room to equilibrate to room temperature before use.
- Turn on plate reader. Wait until "ready"
- Turn on computer and log on using your current Windows password.
- Open and log in to WinKQCL
- Click on the Templates tab and click "New".
- Under "Test Type" select "Routine Assay".
- Click the "Lot/Exp" tab and enter the lot number and expiration of the lysate, water and standard endotoxin to be used in the assay.
- Confirm the concentrations of the points on the standard curve (5 points at 50 – 0.005 EU/ml).
- Ensure that the pipettes, tips, tubes and other accessories are correctly recorded in the accessories section of the assay template in WinKQCL.
- Add the details of the samples to be tested from the master list.
- When the PPC box is ticked a default value will enter. Change this to the correct value.
- Most assays will use a final PPC concentration of 0.5 EU/ml.
- Name the template: Date (DDMmmYY) Operator initials Vaccine, and then Save. The default "Name" is the date of the assay.
- Print Plate Layout in "Landscape" to help set up the dilutions.

Preparation of Control Standard Endotoxin

- CSE dilutions can be dispensed to the plate as they are prepared to save mixing time.
- Open reaction plate from packet
- If required, reconstitute the endotoxin with the volume of LRW specified on the Lonza C of A.
- Vortex vigorously for at least 15 minutes.
- Rack and label dilution tubes/bottles as in the tables below
- Dispense LRW into dilution tubes/bottles

Concentration	Volume LRW	Volume Endotoxin	Plate wells	% CV
50 EU/ml	Direct from vial	-	F1 – F2	2.41
5 EU/ml	900 µl LRW	100 µl of 50 EU/ml	E1 – E2	2.35
0.5 EU/ml	900 µl LRW	100 µl of 5 EU/ml	D1 – D2	2.24
0.05 EU/ml	900 µl LRW	100 µl of 0.5 EU/ml	C1 – C2	1.49
0.005 EU/ml	900 µl LRW	100 µl of 0.05 EU/ml	B1 – B2	4.32
Blank	Direct from vial	-	A1 – A2	

- Immediately after removing the CSE from the vortex, dispense 100 µl of the 50 EU/ml CSE into the appropriate wells of the plate (F1 & F2).
- Pipette 100 µl into the 5 EU/ml tube/bottle as instructed above to make the next dilution.
- Vortex the 5 EU/ml tube/bottle for 1 minute, add 100 µl to the plate (E1 & E2) and 100 µl to the 0.5 EU/ml tube/bottle
- Continue to prepare dilutions and dispense into the reaction plate as instructed above, vortexing for 1 minute between dilutions
- Dispense 100 µl LRW into the appropriate wells of the plate (A1 & A2)

Preparation of Samples

- Retrieve the required samples from cold room to equilibrate to room temperature before use
- Rack and label the appropriate tubes for predilution and testing of samples
- For routine assays a PPC with a final concentration approximating 0.5 EU/ml is used. The 100 µl sample in the PPC wells is “spiked” with 10 µl of the 5 EU/ml standard to obtain a final concentration of 0.45 EU/ml.
- Vortex the 5 EU/ml standard for 1 minute
- Pipette 10 µl of the 5 EU/ml standard to the appropriate PPC wells as per the plate layout
- Remove the label from the first sample and place it on the corresponding Sample Results Sheet
- Vortex the first sample on full speed for 10-20 seconds
- Transfer sample to the appropriate tube/bottle labelled “Neat”
- Prepare sample dilutions as in table in Appendix 1K, vortexing for 5 seconds before transferring to the next dilution tube. Repeat until all dilutions of the first sample are prepared.
- Dispense 100 µl of the final dilution into the 4 appropriate wells as per the plate layout
- Repeat with remaining samples

Starting the Assay

- The plate is then ready for the reaction. Prepare the software for the reaction as follows:
- Return to “Templates” screen
- Select the correct Available Template, Add it to the Merged Template field and click “Run”
- WinKQCL will then ask which plate reader you wish to use. Select the ELx808 and click OK.
- Pre-warming. Leave the lid of the plate on, place the plate into the reader and click OK, this starts the 10 minute warm-up
- Reconstitute the required lysate vial/s with the specified volume of LRW stated on the vial
- Gently swirl /roll the vial to reconstitute lysate. Avoid frothing.
- Immediately before use, pour the lysate from the vial/s into the reagent reservoir, removing the final drops with a pipette
- Open cover
- Add 100 µl lysate to each of the assay wells, carefully, and as quickly as possible
- Close the cover on the plate reader and click OK to start the run. **Do not open cover.**

Acceptance Criteria

The CSE standard curve must meet the following parameters for the assay to be considered valid.

- Correlation coefficient (r) absolute value ≥ 0.980
- Slope between -0.400 and -0.100
- Y intercept between 2.500 and 3.500
- Mean reaction times of blank ≥ mean reaction times of lowest standard
- Coefficient of variation (CV) values for all standards are < 10%

-0.996  
-0.217  
3.126  
Yes  
Yes

Were all acceptance criteria for the assay met ?

Yes / No

Conclusions

- Prior to printing, the Operator should electronically sign the report and record any deviations from the method during the e-signature procedure
- Transcribe results to Assay Worksheet and Sample Result Sheet/s i.e. % CV's, acceptance criteria parameters, EU/ml, and % PPC Recovery
- Have another operator, preferably the person responsible for endotoxin testing, record any explanation of unexpected outcomes and sign electronically as a “Reviewer”

Record Details	D17-686127 Bacterial Endotoxin Testing - Appendix 2K - Kinetic LAL Routine Assay Worksheet.DOCX		
Last Editor	s22	Edit Date	22/08/2017 11:23 AM
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The results are stored as part of the assay in the WinKQCL software. Printed versions are stored in Endotoxin Testing Results folders in FC33.

Notes

Record Details	D17-686127 Bacterial Endotoxin Testing - Appendix 2K - Kinetic LAL Routine Assay Worksheet.DOCX		
Last Editor	s22	Edit Date	22/08/2017 11:23 AM
Print Date	29/06/2021 12:17 PM		Page 4 of 4



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Laboratories Branch

Operations	Bacterial Endotoxin Manual
Procedure	Bacterial Endotoxin Testing - Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet
Written	s22
Authorised	s22
Date issued	7 April 2021
Revision #	3

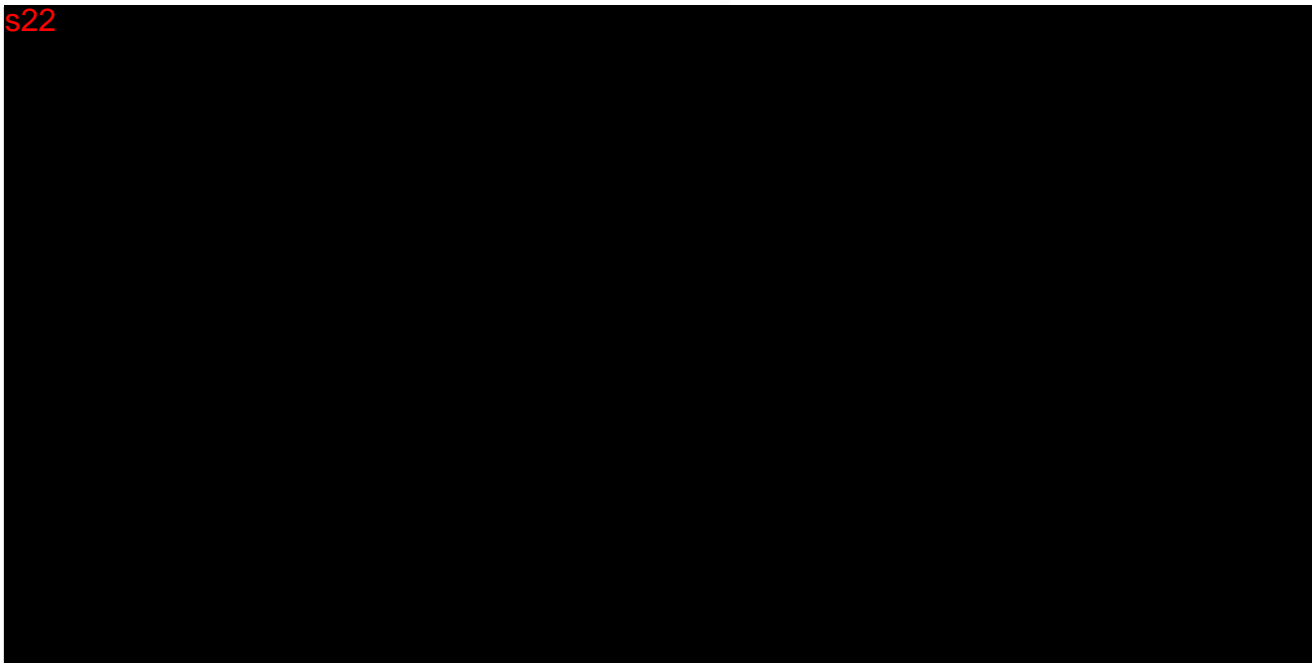
Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 29JUN21/1 Operator: s22  
 (format DDMMYY/1)

LAL Reagent Water (LRW) Lot Number: 0000830072 LRW Expiry: 01MAR2022

Other Reagent: - Batch# - Expiry: -

Product Details



For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Were the standard curve validity criteria met? Yes / No  
 Were the sample validity criteria met? Yes / No  
 Was the result within the endotoxin limit? Yes / No

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

Record Details	D21-2432168 Bacterial Endotoxin Testing - Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet DOCM		
Last Editor	s22	Edit Date	7/04/2021 10:44 AM
Print Date	29/06/2021 12:20 PM		Page 1 of 1



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Operations	Bacterial Endotoxin Manual
Procedure	Bacterial Endotoxin Testing - Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet
Written	s22
Authorised	s22
Date issued	7 April 2021
Revision #	3

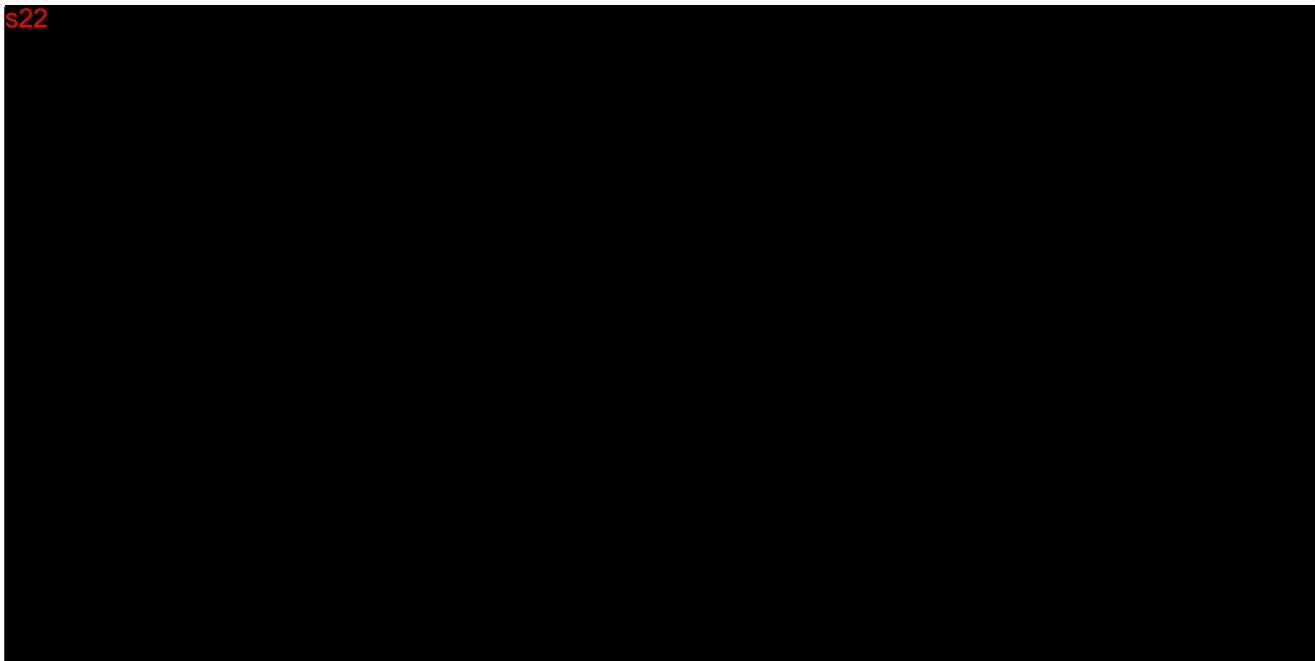
Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 29JUN21/1 Operator: s22  
(format DDMMYY/1)

LAL Reagent Water (LRW) Lot Number: 0000830072 LRW Expiry: 31MAR2022

Other Reagent: — Batch# — Expiry: —

Product Details



For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Were the standard curve validity criteria met ? Yes / No  
 Were the sample validity criteria met ? Yes / No  
 Was the result within the endotoxin limit ? Yes / No

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

Record Details	D21-2432168 Bacterial Endotoxin Testing - Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet DOCM		
Last Editor	s22	Edit Date	7/04/2021 10:44 AM
Print Date	29/06/2021 12:20 PM		Page 1 of 1



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Laboratories Branch

Operations	Bacterial Endotoxin Manual
Procedure	Bacterial Endotoxin Testing - Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet
Written	s22
Authorised	s22
Date issued	7 April 2021
Revision #	3

### Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

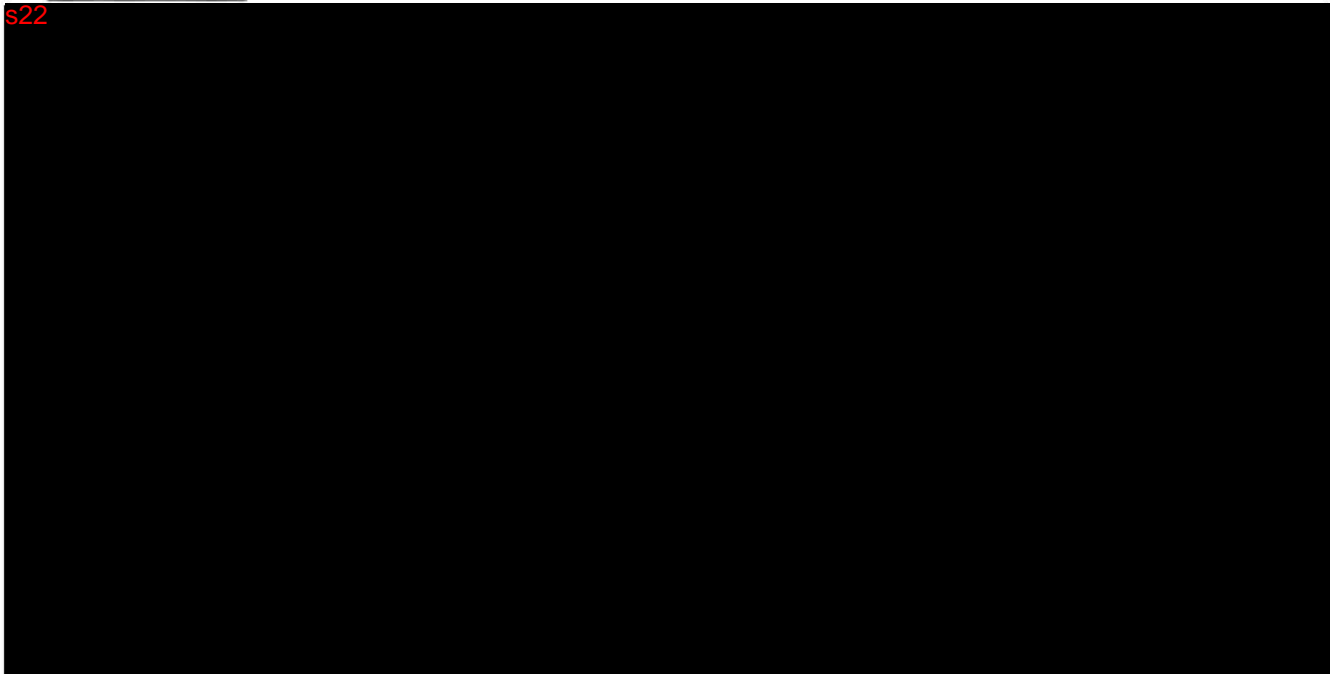
Assay ID: 29JUN21/1 Operator: s22  
 (format DDMMYY/1)

LAL Reagent Water (LRW) Lot Number: 0000830072 LRW Expiry: 01 MAR 2022

Other Reagent: PYROSPERSE Batch# 0000843500 Expiry: 2 OCT 2021

USE BY: 23 JUL 2021

#### Product Details



For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Were the standard curve validity criteria met ?  Yes /  No

Were the sample validity criteria met ?  Yes /  No

Was the result within the endotoxin limit ?  Yes /  No

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

Record Details	D21-2432168 Bacterial Endotoxin Testing - Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet.DOCM		
Last Editor	s22	Edit Date	7/04/2021 10:44 AM
Print Date	29/06/2021 12:20 PM		Page 1 of 1





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Laboratories Branch

Operations	Bacterial Endotoxin Manual
Procedure	Bacterial Endotoxin Testing - Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet
Written	s22
Authorised	s22
Date issued	7 April 2021
Revision #	3

Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 29JUN21/1 Operator: s22  
 (format DDMMYY/1)

LAL Reagent Water (LRW) Lot Number: 0000830072 LRW Expiry: 01MAR2022

Other Reagent: PYROSPERSE Batch# 0000843500 Expiry: 2OCT2021  
 USE BY: 23JUL2021

Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>COMIRNATV</u>	<u>FE3064</u>	<u>10/2021</u>	<u>2106002467-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
<u>-</u>	<u>s47</u>	<u>2500</u>	<u>1000</u>

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>1/50</u>	<u>20µl of vaccine</u>	<u>975µl</u>	<u>5 µl</u>
<u>for 1/1000: 1/20</u>	<u>50 µl of 1/50 diln.</u>	<u>945µl</u>	<u>5 µl</u>

Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay paperwork.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>s47</u>	<u>[REDACTED]</u>	<u>0.63</u>	<u>117</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

- Were the standard curve validity criteria met? Yes / No
- Were the sample validity criteria met? Yes / No
- Was the result within the endotoxin limit? Yes / No

This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.

Record Details	D21-2432168 Bacterial Endotoxin Testing - Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet DOCM		
Last Editor	<u>s22</u>	Edit Date	7/04/2021 10:44 AM
Print Date	29/06/2021 12:20 PM		Page 1 of 1



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Laboratories Branch

Operations	
Procedure	Bacterial Endotoxin Testing - Appendix 4K – Kinetic Control Standard Endotoxin (CSE) Preparation
Written	s22
Authorised	s22
Date issued	16 August 2017
Revision #	1

### Appendix 4K – Kinetic Control Standard Endotoxin (CSE) Preparation

CSE Lot No: 0000820346

CSE expiry date: 01/07/2023

Refer to website of the manufacturer to download the Certificate of Analysis. This will report the correct reconstitution volume. You will need to enter the Catalogue Number (50-650U) and the Lot Number located on the kit box.

The contents of the vial are under vacuum. Remove the vial stopper slowly and carefully to avoid the loss of lyophilised powder.

Reconstitute CSE with: 3.7 ml of LAL Reagent Water (4 x 925 µl)

LRW Batch Number: 0000830072

Date reconstituted: 29 JUN 2021

Operator(s): s22

Reconstituted expiry date: 27 JULY 2021 (Lonza KQCL- 28 days)

CSE is a specialised biological preparation. For the kinetic assay, the CSE is reconstituted to ensure a starting endotoxin content of 50 EU/ml. The endotoxin potency of each batch of CSE is variable. The manufacturer determines the endotoxin content (EU/ng) using the Reference Standard Endotoxin (RSE) and reports the appropriate reconstitution volume.

Label the CSE vial with the reconstitution date and the expiry date. This is **28 days** after reconstitution when stored at 2-8°C.

Secure the reconstituted CSE to a vortex mixer and shake vigorously for a minimum of 15 minutes before each use.

Record Details	D:\17_686184_Bacterial Endotoxin Testing - Appendix 4K - Kinetic CSE Preparation.DOCX		
Last Editor	s22	Edit Date	22/08/2017 11:22 AM
Print Date	29/06/2021 12:16 PM		Page 1 of 1



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Laboratories Branch

Operations	
Procedure	Bacterial Endotoxin Testing - Appendix 3K – Kinetic Lysate Preparation
Written	s22
Authorised	s22
Date issued	16 August 2017
Revision #	1

**Appendix 3K – Kinetic Lysate Preparation**

Vials of lysate should only be reconstituted during the last few minutes of the plate equilibration step. The reconstituted lysate should only be dispensed into a pyrogen free reagent reservoir immediately before use.

Lysate Lot No: WLO29ITCAW

Lysate expiry date: 21 OCT 2022

Check whether the sensitivity of this batch of lysate has been confirmed previously.

Date lysate sensitivity confirmed: 01 JUN 2021

**Refer to manufacturer's instructions and lysate vial for reconstitution volume**

**The contents of the vial are under vacuum. Remove the vial stopper slowly and carefully to avoid the loss of lyophilised powder.**

Reconstitute lysate with: 2.6 ml of LAL Reagent Water (LRW) (3 x 867 µl)

LRW Batch Number: 0000830072

Date reconstituted: 29 JUN 2021

Operator(s): s22

Use by date: 13 July 2021 (Lonza KQCL- 14 days from reconstitution)

Swirl vial gently **without** vigorous shaking until dissolution of lyophilised lysate is complete (at least 30 seconds). After reconstitution, **and only immediately before use**, pour into a pyrogen free reagent reservoir.

If using on the same day, store at 2-8°C protected from light until ready for use.

Excess quantities of the lysate can be stored in the original vial protected from light at below -10°C (usually -80°C) until the reconstituted expiry date of **14 days**.

Record Details	D17-686156 Bacterial Endotoxin Testing - Appendix 3K - Kinetic Lysate Preparation.DOCX		
Last Editor	s22	Edit Date	22/08/2017 11:22 AM
Print Date	29/06/2021 12:16 PM	Page 1 of 1	

**Biotherapeutics Section, TGA - Biotherapeutics**

Displayed in Version: 6.0.2

Report Generated: 29/06/2021 4:50:51PM (UTC+10)

**SpectraMax M5 reader**

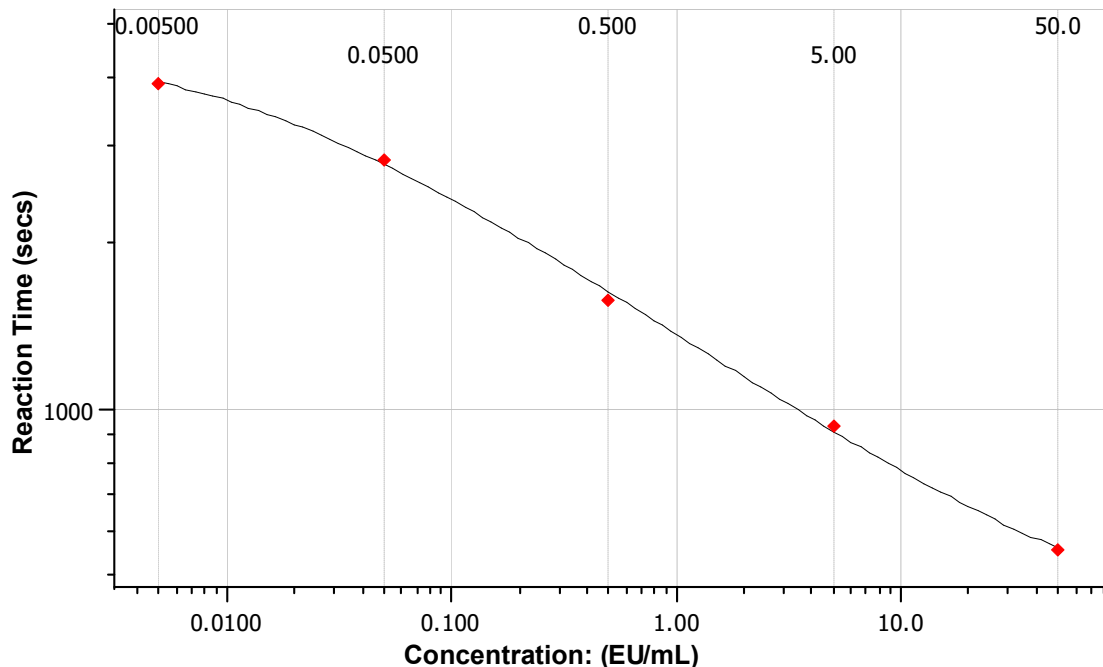
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**Lonza - WinKQCL Version 6.0.2**

Page 1 of 4

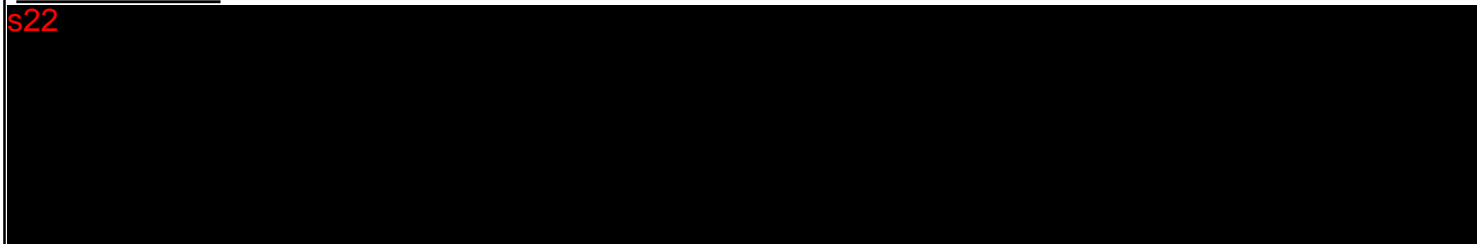
Performed By : <b>s22</b>	LAL Lot No.:	WL029JJCAW	Exp : 21/10/2022	Time : 3:14:38PM (UTC+10)
KQCL - Routine Test	Water Lot No.:	0000830072	Exp : 1/03/2022	Date : 29/06/2021
Template : 2021 Jun 29 - <b>s22</b> Pfizer	Endotoxin Lot No.:	0000820346	Exp : 1/07/2023	SPECTRAmax M5
Covid Vaccines				S/N mv05705
Temperature : 36.7°C - 37.0°C				
Linear Regression :	Correlation Coefficient = -0.996	Slope = -0.217	Y-Intercept = 3.126	
PowerCurve :	A = 3.1356	B = -0.2557	C = -0.0012	D = 0.0104
				E = 0.0000



**Standards Results**

Standards	Conc./Dil.	Well	Reaction Time (sec)	Average Reaction Time (sec)	Back Prediction (Power Curve)
Blank	Blank	A 1	4065	4098	< 0.00500
		A 2	4130		
Std. 1	0.00500	B 1	3765	3884	0.00533
		B 2	4002		
Std. 2	0.0500	C 1	2810	2840	0.0446
		C 2	2870		
Std. 3	0.500	D 1	1551	1576	0.571
		D 2	1601		
Std. 4	5.00	E 1	917	933	4.56
		E 2	948		
Std. 5	50.0	F 1	548	558	51.2
		F 2	567		

**Product Results**



Signed By : **s22** (et1-sig) Date/Time : 29/06/2021 4:50:16PM(UTC+10)

Reviewed By : **s22** (et1-sig) Date/Time : 29/06/2021 4:50:49PM(UTC+10)

( !! = Masked, \*\*\*\* = reaction time > 5550, ??? = atypical, # = Modified, >>>> = High OD, See Comment Page)

( In Notes : ! = Masked Point(s), \* = Point(s) Did Not React, ? = Atypical Point(s), # = Modified, > = High OD, <LS = Less than the lowest standard)

**Biotherapeutics Section, TGA - Biotherapeutics**

Displayed in Version: 6.0.2

Report Generated: 29/06/2021 4:50:52PM (UTC+10)

**SpectraMax M5 reader**

Document 31  
Calculated in Version: 6.0.2

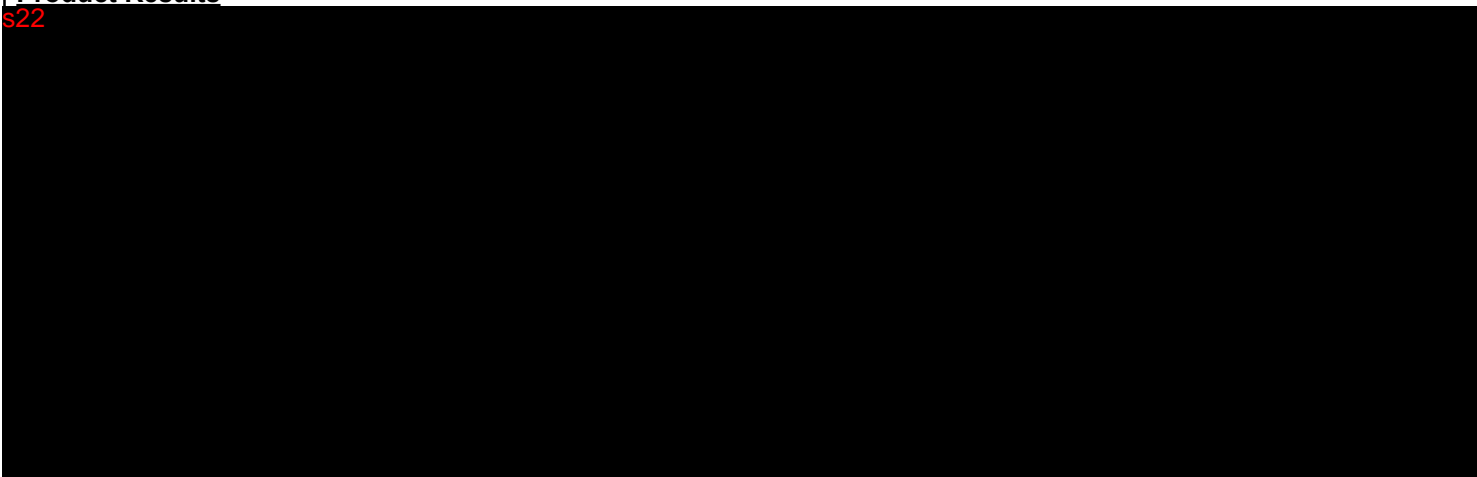
Results Generated: 29/06/2021 4:48:30PM (UTC+10)

**Lonza - WinKQCL Version 6.0.2**

Page 2 of 4

Performed By : <b>s22</b>	LAL Lot No.:	WL029JJCAW	Exp : 21/10/2022	Time : 3:14:38PM (UTC+10)
KQCL - Routine Test	Water Lot No.:	0000830072	Exp : 1/03/2022	Date : 29/06/2021
Template : 2021 Jun 29 - <b>s2</b> Pfizer	Endotoxin Lot No.:	0000820346	Exp : 1/07/2023	SPECTRAmax M5
Covid Vaccines				S/N mv05705
Temperature : 36.7°C - 37.0°C				
Linear Regression :	Correlation Coefficient = -0.996	Slope = -0.217	Y-Intercept = 3.126	
PowerCurve :	A = 3.1356	B = -0.2557	C = -0.0012	D = 0.0104
				E = 0.0000

**Product Results**



Unique Id: 000289-001158		Pfizer Covid vaccine - Comirnaty		Lot No. FE3064	ID. 2106002467		
Samples	Dilution	Well	Reaction Time (sec)	Average Reaction Time (sec)	Raw EU	Results (Power Curve) EU/mL	Release Limit
S1	1000	E 3	5404	5402	<b>s47</b>	<b>s47</b>	<b>s47</b>
		E 4	5400				
PPC	1000	F 3	1573	1566	0.586		
		F 4	1559				
PPC Value: 0.5		% PPC Recovery :		117%	(PPC - SAMPLE 1) Endotoxin Recovered : 0.586		

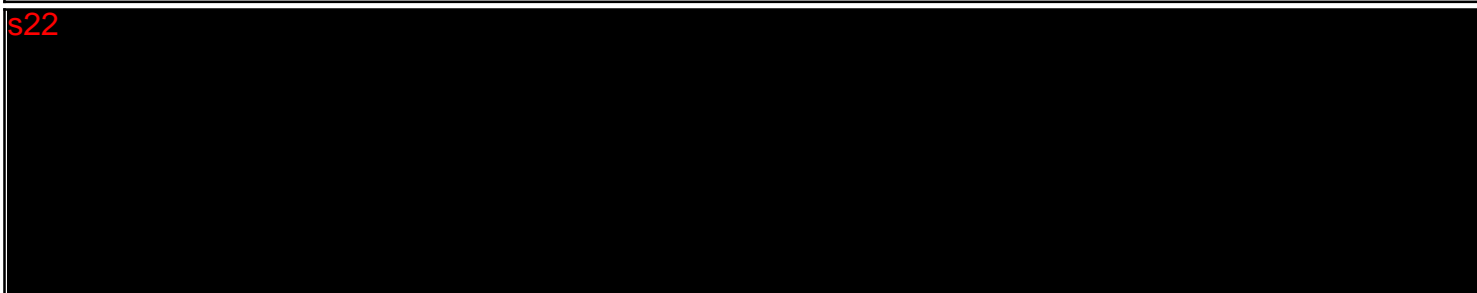
**Curve Parameters**

Parameter	Value	Specification	Status
Correlation Coefficient	-0.996	-1.000 to -0.980	PASS
Slope	-0.217	-0.400 to -0.100	PASS
Y Intercept	3.126	2.500 to 3.500	PASS

**Standards AER**

Concentration	CV	Specification	Status	Notes
0.00500	4.32%	<10.00%	PASS	
0.0500	1.49%	<10.00%	PASS	
0.500	2.24%	<10.00%	PASS	
5.00	2.35%	<10.00%	PASS	
50.0	2.41%	<10.00%	PASS	

Blank/lowest standard alert based on average replicates.



Signed By : **s22** (et1-sig) Date/Time : 29/06/2021 4:50:16PM(UTC+10)

Reviewed By : **s22** (et1-sig) Date/Time : 29/06/2021 4:50:49PM(UTC+10)

( !! = Masked, \*\*\*\* = reaction time > 5550, ???? = atypical, # = Modified, >>>> = High OD, See Comment Page)

( In Notes : ! = Masked Point(s), \* = Point(s) Did Not React, ? = Atypical Point(s), # = Modified, > = High OD, <LS = Less than the lowest standard)

**Biotherapeutics Section, TGA - Biotherapeutics**

Displayed in Version: 6.0.2

Report Generated: 29/06/2021 4:50:52PM (UTC+10)

**SpectraMax M5 reader**

Calculated in Version: 6.0.2

Results Generated: 29/06/2021 4:48:30PM (UTC+10)

**Lonza - WinKQCL Version 6.0.2**

Page 3 of 4

Performed By : <b>s22</b>	LAL Lot No.:	WL029JJCAW	Exp : 21/10/2022	Time : 3:14:38PM (UTC+10)
KQCL - Routine Test	Water Lot No.:	0000830072	Exp : 1/03/2022	Date : 29/06/2021
Template : 2021 Jun 29 - AZ & Pfizer	Endotoxin Lot No.:	0000820346	Exp : 1/07/2023	SPECTRAmax M5
Covid Vaccines				S/N mv05705
Temperature : 36.7°C - 37.0°C				
Linear Regression :	Correlation Coefficient = -0.996	Slope = -0.217	Y-Intercept = 3.126	
PowerCurve :	A = 3.1356	B = -0.2557	C = -0.0012	D = 0.0104
				E = 0.0000


**Product AER**

Concentration/ Dilution	Parameter	Results (Power Curve)	Specification	Status	Notes
<b>s22</b>					
<b>s22</b>					
<b>Pfizer Covid vaccine - Comirnaty — Lot No. FE3064 ID. 2106002467</b>					
1000	%CV	<b>s47</b>	<b>s47</b>	PASS	
	Endotoxin			PASS	
PPC	%CV	0.63%	< 10.00%	PASS	
PPC	% PPC Recovery	117%	50 to 200	PASS	

**Reader Parameters:**

Delta t (s) = 150	Measurement Filter (nm) = 405	Delta mOD = 200
Shake (s) = 30	Actual/Max Reads = 38/40	

**Accessories Summary**

Name	Comment	Lot No.	Manufacturer	Cal. Due Date	Exp
Biopur Blue Tips		H177688K-2215	Eppendorf		28/05/2023
Biopur Yellow Tips		G174443O - 1340	Eppendorf		30/09/2022
LAL reagent water		0000830072	Lonza		1/03/2022
Used for reconstituted CSE on 1/06/2021					
Multichannel Pipette		32199	Eppendorf	21/09/2021	
Cal: 07Jun2021					
Pipette (100-1000)		5707	Eppendorf	19/08/2021	
Pipette (20-200)		32832	eppendorf	18/07/2021	
Cal 13Apr 2021					
Pipette (2-20)		32704	eppendorf	30/07/2021	
Cal: 13Apr2021					
Plate		12919601	costar		8/05/2022
Pyrogen free tubes		0000891400	Lonza		31/03/2024
Pyrospense		0000843500	LONZA		2/10/2021
Reagent reservoirs		4215899	Lonza		27/07/2023

Signed By : **s22** (et1-sig) Date/Time : 29/06/2021 4:50:16PM(UTC+10)

Reviewed By : **s22** (et1-sig) Date/Time : 29/06/2021 4:50:49PM(UTC+10)

( !! = Masked, \*\*\*\* = reaction time > 5550, ??? = atypical, # = Modified, >>>> = High OD, See Comment Page)

( In Notes : ! = Masked Point(s), \* = Point(s) Did Not React, ? = Atypical Point(s), # = Modified, > = High OD, <LS = Less than the lowest standard)

**Biotherapeutics Section, TGA - Biotherapeutics**

Displayed in Version: 6.0.2

Report Generated: 29/06/2021 4:50:52PM (UTC+10)

**SpectraMax M5 reader**

Document 31  
Calculated in Version: 6.0.2

Results Generated: 29/06/2021 4:48:30PM (UTC+10)

**Lonza - WinKQCL Version 6.0.2**

Page 4 of 4

Performed By : s22	LAL Lot No.:	WL029JJCAW	Exp : 21/10/2022	Time : 3:14:38PM (UTC+10)
KQCL - Routine Test	Water Lot No.:	0000830072	Exp : 1/03/2022	Date : 29/06/2021
Template : 2021 Jun 29 - s22 Pfizer	Endotoxin Lot No.:	0000820346	Exp : 1/07/2023	SPECTRAmax M5
Covid Vaccines				S/N mv05705
Temperature : 36.7°C - 37.0°C				
Linear Regression :	Correlation Coefficient = -0.996	Slope = -0.217	Y-Intercept = 3.126	
PowerCurve :	A = 3.1356	B = -0.2557	C = -0.0012	D = 0.0104
				E = 0.0000

**Log Summary**

Description	Comment	User ID	Date
Routine Test RunDateTime: 29/06/2021 3:14:38 PM (AUS Eastern Standard Time) Serial # : mv05705		s22	29/06/2021 3:14:38PM
The polynomial of order 4 is invalid because it is not monotonic (slope must be always negative); Routine Test RunDateTime: 29/06/2021 3:14:38 PM (AUS Eastern Standard Time) Serial # : mv05705		s22	29/06/2021 4:48:29PM
Analyst E-sig applied - s22 Run Date/Time - Jun 29 2021 03:14:38 PM (AUS Eastern Standard Time) Number - 0		s22	29/06/2021 4:50:16PM
Reviewer E-sig applied - s22 Run Date/Time - Jun 29 2021 03:14:38 PM (AUS Eastern Standard Time) Number - 0		s22	29/06/2021 4:50:49PM

Signed By : s22 (et1-sig) Date/Time : 29/06/2021 4:50:16PM(UTC+10)

Reviewed By : s22 (et1-sig) Date/Time : 29/06/2021 4:50:49PM(UTC+10)

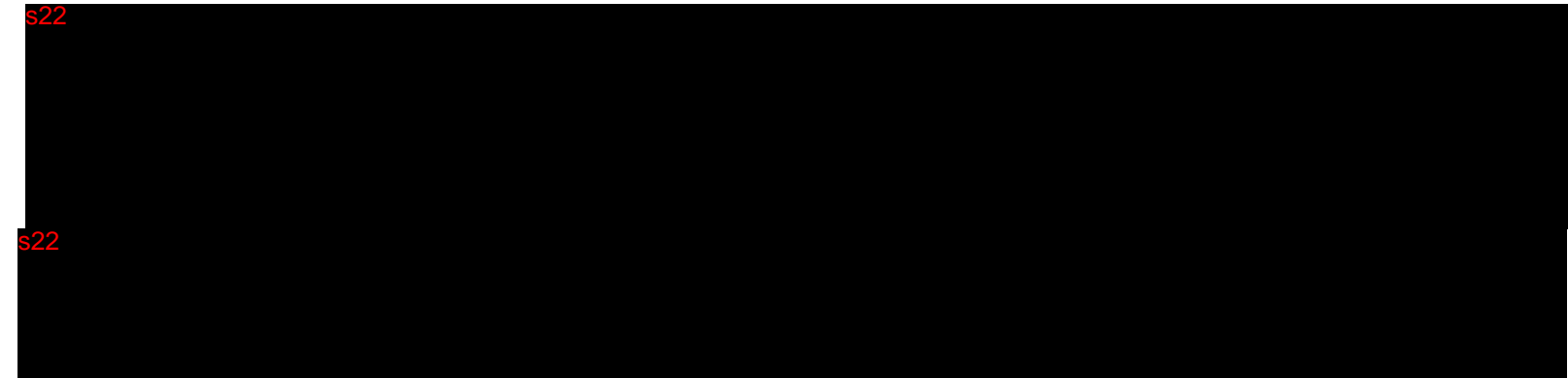
( !! = Masked, \*\*\*\* = reaction time > 5550, ??? = atypical, # = Modified, >>>> = High OD, See Comment Page)

( In Notes : ! = Masked Point(s), \* = Point(s) Did Not React, ? = Atypical Point(s), # = Modified, > = High OD, <LS = Less than the lowest standard)

Data from Smear Analysis Table

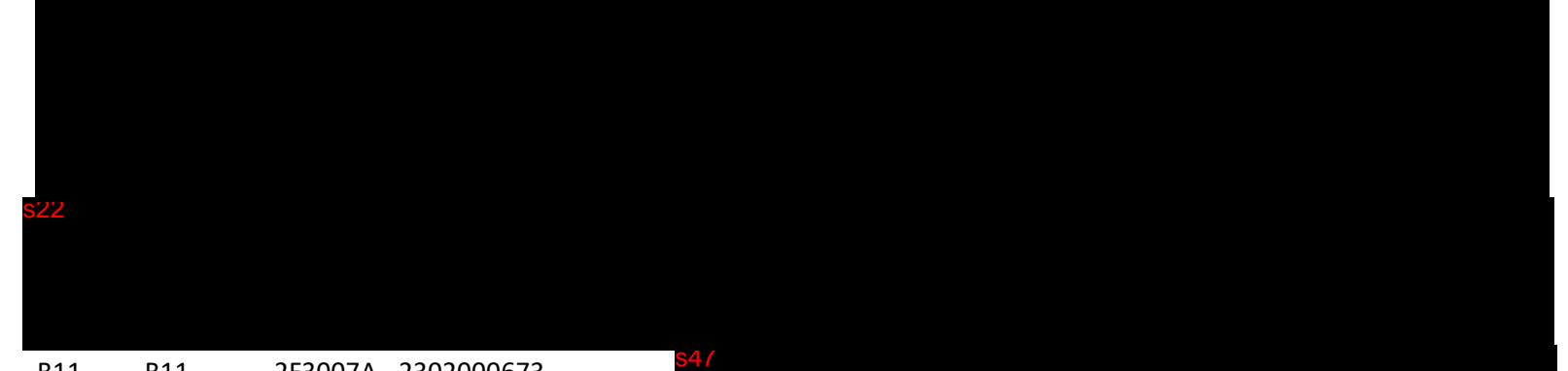
1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate  
 2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the two smear sets.  
 3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	A1	Blank	3700 nt to 4800 nt	0.0043	55.5	0.0032	4160	4.04
A1	A1	Blank	4800 nt to 13000 nt	0.0003	4.4	0.0001	11596	7.09
A2	A2	Blank	3700 nt to 4800 nt	0.0012	26.7	0.001	3963	2.71
A2	A2	Blank	4800 nt to 13000 nt	0	0.4	0	11068	6.02
A3	A3	Blank	3700 nt to 4800 nt	0.0024	45.7	0.0019	3930	2.6
A3	A3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A4	A4	Blank	3700 nt to 4800 nt	0.0018	44.5	0.0014	3912	2.87
A4	A4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A5	A5	Blank	3700 nt to 4800 nt	0.0009	1.5	0.0007	3979	1.45
A5	A5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN



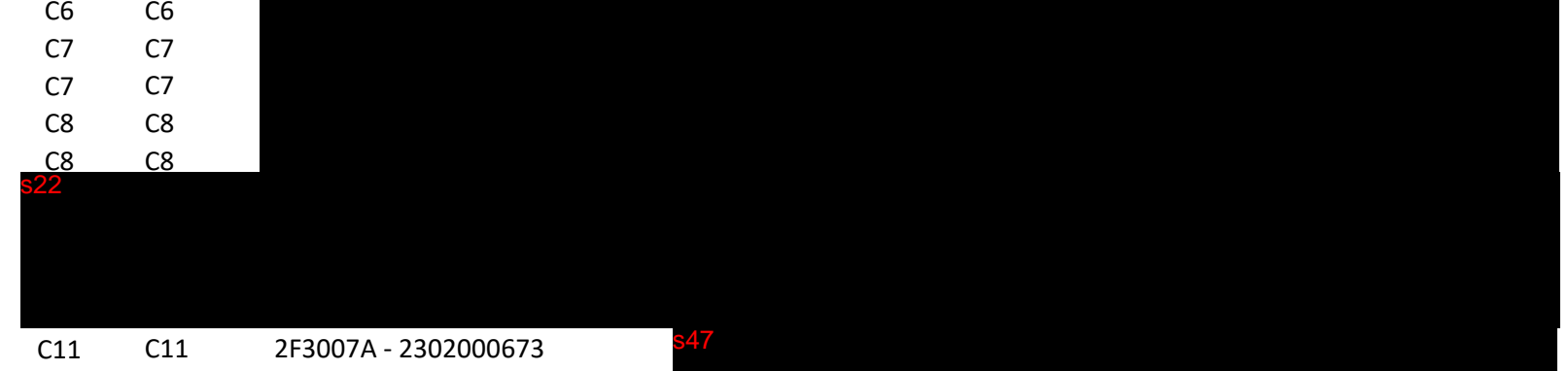
A11	A11	2F3007A - 2302000673						
A11	A11	2F3007A - 2302000673						

B1	B1	Blank	3700 nt to 4800 nt	0.0031	42.4	0.0022	4382	2.98
B1	B1	Blank	4800 nt to 13000 nt	0.0001	1.2	0	12014	6.45
B2	B2	Blank	3700 nt to 4800 nt	0.0241	3	0.0171	4407	7.67
B2	B2	Blank	4800 nt to 13000 nt	0.3439	43.3	0.1235	8688	27.3
B3	B3	Blank	3700 nt to 4800 nt	0.0014	21.4	0.0009	4445	3.34
B3	B3	Blank	4800 nt to 13000 nt	0	0.7	0	4825	0.27
B4	B4	Blank	3700 nt to 4800 nt	0.004	50.6	0.003	4195	4.41
B4	B4	Blank	4800 nt to 13000 nt	0	0.1	NaN	NaN	NaN
B5	B5	Blank	3700 nt to 4800 nt	0.0014	24	0.001	4418	6.81
B5	B5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN



B11	B11	2F3007A - 2302000673						
B11	B11	2F3007A - 2302000673						

C1	C1	Blank	3700 nt to 4800 nt	0.0048	34.3	0.0034	4434	5.84
C1	C1	Blank	4800 nt to 13000 nt	0.0031	22.3	0.0019	4968	3.57
C2	C2	Blank	3700 nt to 4800 nt	0.0007	21.2	0.0005	4504	5.03
C2	C2	Blank	4800 nt to 13000 nt	0.0001	4.2	0.0001	5038	7.51
C3	C3	Blank	3700 nt to 4800 nt	0.0018	22	0.0013	4420	3.86
C3	C3	Blank	4800 nt to 13000 nt	0.0013	15	0.0006	7028	47.11
C4	C4	Blank	3700 nt to 4800 nt	0.0008	44.6	0.0006	4531	2.26
C4	C4	Blank	4800 nt to 13000 nt	0.0001	4.2	0	5060	0.96
C5	C5	Blank	3700 nt to 4800 nt	0.0018	63.7	0.0012	4501	2.52
C5	C5	Blank	4800 nt to 13000 nt	0.0002	6.1	0.0001	4914	1.01



C11	C11	2F3007A - 2302000673						
C11	C11	2F3007A - 2302000673						

D1	D1	Blank	3700 nt to 4800 nt	0.0037	33.5	0.0027	4324	4.22
D1	D1	Blank	4800 nt to 13000 nt	0.0005	4.3	0.0001	11006	22.16
D2	D2	Blank	3700 nt to 4800 nt	0.0045	40.3	0.0034	4207	4.25
D2	D2	Blank	4800 nt to 13000 nt	0.0003	2.3	0.0001	12513	8.21
D3	D3	Blank	3700 nt to 4800 nt	0.0007	14.7	0.0005	4189	2.86
D3	D3	Blank	4800 nt to 13000 nt	0.0003	5.8	0.0001	12377	3.54
D4	D4	Blank	3700 nt to 4800 nt	0.001	22.1	0.0008	4116	3.13
D4	D4	Blank	4800 nt to 13000 nt	0.0002	4.6	0.0001	11097	24.3
D5	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D6	D6	Blank	3700 nt to 4800 nt	0.0006	19	0.0005	4017	2.85
D6	D6	Blank	4800 nt to 13000 nt	0.0003	10.2	0.0001	11321	18.82
D7	D7	Blank	3700 nt to 4800 nt	0.0005	5.2	0.0004	3944	1.65
D7	D7	Blank	4800 nt to 13000 nt	0.0003	3.6	0.0001	11666	8.76
D8	D8	Blank	3700 nt to 4800 nt	0.0004	0.2	0.0003	4004	1.37
D8	D8	Blank	4800 nt to 13000 nt	0	0	0	10564	21.79
D9	D9	Blank	3700 nt to 4800 nt	0.2027	2.5	0.1496	4228	7.77
D9	D9	Blank	4800 nt to 13000 nt	0.8063	10	0.2867	8776	27.01
D10	D10	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D10	D10	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D11	D11	Blank	3700 nt to 4800 nt	0.0008	0.3	0.0006	3927	5.08
D11	D11	Blank	4800 nt to 13000 nt	0.0018	0.7	0.0006	9461	30.66
D12	D12	Ladder						



OFFICIAL

Q-Pulse Document Number	Bio-BPC-Form-13
Title	Fragment Analyzer Spreadsheet - 2 smears
Author	s22
Owner (Reviewer)	s22
Approver (Authoriser)	s22
Date Authorised	23/09/2022
Revision Number	2

Analyst	s22
Assay Date	17/03/2023

Product specific parameters for Pass/Fail		
minimum	cut off	maximum
s47		
result >>	s47	

							% INTEGRITY SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	Sample ID	Average	stdev	%CV	
1	A1	Blank	3700 nt to 4800 nt	55.5	4160	4.04	Blank	44.07	10.70	24.28	FAIL
2	B1	Blank	3700 nt to 4800 nt	42.4	4382	2.98					
3	C1	Blank	3700 nt to 4800 nt	34.3	4434	5.84					
1	A2	Blank	3700 nt to 4800 nt	26.7	3963	2.71	Blank	16.97	12.40	73.11	FAIL
2	B2	Blank	3700 nt to 4800 nt	3	4407	7.67					
3	C2	Blank	3700 nt to 4800 nt	21.2	4504	5.03					
1	A3	Blank	3700 nt to 4800 nt	45.7	3930	2.6	Blank	29.70	13.86	46.67	FAIL
2	B3	Blank	3700 nt to 4800 nt	21.4	4445	3.34					
3	C3	Blank	3700 nt to 4800 nt	22	4420	3.86					
1	A4	Blank	3700 nt to 4800 nt	44.5	3912	2.87	Blank	46.57	3.49	7.50	FAIL
2	B4	Blank	3700 nt to 4800 nt	50.6	4195	4.41					
3	C4	Blank	3700 nt to 4800 nt	44.6	4531	2.26					
1	A5	Blank	3700 nt to 4800 nt	1.5	3979	1.45	Blank	29.73	31.49	105.92	FAIL
2	B5	Blank	3700 nt to 4800 nt	24	4418	6.81					
3	C5	Blank	3700 nt to 4800 nt	63.7	4501	2.52					
s22											
s22											
1	A11	2F3007A - 2302000673		s47			2F3007A - 2302000673	s47	s47	s47	PASS
2	B11	2F3007A - 2302000673									
3	C11	2F3007A - 2302000673									
s22											
1	D1	Blank	3700 nt to 4800 nt	33.5	4324	4.22	Blank	29.50	13.26	44.95	FAIL
2	D2	Blank	3700 nt to 4800 nt	40.3	4207	4.25					
3	D3	Blank	3700 nt to 4800 nt	14.7	4189	2.86					
1	D4	Blank	3700 nt to 4800 nt	22.1	4116	3.13	Blank	13.70	11.97	87.34	FAIL
2	D5	Blank	3700 nt to 4800 nt	0	NaN	NaN					
3	D6	Blank	3700 nt to 4800 nt	19	4017	2.85					
1	D7	Blank	3700 nt to 4800 nt	5.2	3944	1.65	Blank	2.63	2.50	95.04	FAIL
2	D8	Blank	3700 nt to 4800 nt	0.2	4004	1.37					
3	D9	Blank	3700 nt to 4800 nt	2.5	4228	7.77					

							% LATE MIGRATING SPECIES SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	Sample ID	Average	stdev	%CV	
1	A1	Blank	4800 nt to 13000 nt	4.4	11596	7.09	Blank	9.30	11.37	122.27	
2	B1	Blank	4800 nt to 13000 nt	1.2	12014	6.45					
3	C1	Blank	4800 nt to 13000 nt	22.3	4968	3.57					
1	A2	Blank	4800 nt to 13000 nt	0.4	11068	6.02	Blank	15.97	23.75	148.73	
2	B2	Blank	4800 nt to 13000 nt	43.3	8688	27.3					
3	C2	Blank	4800 nt to 13000 nt	4.2	5038	7.51					
1	A3	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	5.23	8.47	161.76	
2	B3	Blank	4800 nt to 13000 nt	0.7	4825	0.27					
3	C3	Blank	4800 nt to 13000 nt	15	7028	47.11					
1	A4	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	1.43	2.40	167.20	
2	B4	Blank	4800 nt to 13000 nt	0.1	NaN	NaN					
3	C4	Blank	4800 nt to 13000 nt	4.2	5060	0.96					
1	A5	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	2.03	3.52	173.21	
2	B5	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C5	Blank	4800 nt to 13000 nt	6.1	4914	1.01					
s22											
s22											

§22												
1	A11	2F3007A - 2302000673		§47								
2	B11	2F3007A - 2302000673		2F3007A - 2302000673								
3	C11	2F3007A - 2302000673										
§22												
1	D1	Blank	4800 nt to 13000 nt	4.3	11006	22.16						
2	D2	Blank	4800 nt to 13000 nt	2.3	12513	8.21	Blank	4.13	1.76	42.48		
3	D3	Blank	4800 nt to 13000 nt	5.8	12377	3.54						
1	D4	Blank	4800 nt to 13000 nt	4.6	11097	24.3						
2	D5	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	4.93	5.11	103.54		
3	D6	Blank	4800 nt to 13000 nt	10.2	11321	18.82						
1	D7	Blank	4800 nt to 13000 nt	3.6	11666	8.76						
2	D8	Blank	4800 nt to 13000 nt	0	10564	21.79	Blank	4.53	5.06	111.73		
3	D9	Blank	4800 nt to 13000 nt	10	8776	27.01						

This tab is only to be used if a replicate needs to be excluded from the analysis.

Enter raw data directly into this table. All averages/ pass fail will be calculated automatically using the pass/fail parameters entered in the Calculations tab.

REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS	
							Sample ID	Average	stdev	%CV		
1							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

Product specific parameters for Pass/Fail		
Pass/Fail Parameters		
minimum	cut off	maximum
\$47		
result >>	\$47	

REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
							Sample ID	Average	stdev	%CV	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	



VALIDATION DATA						
REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV
1	A1	sample1-rep1	3500 nt to 5389 nt	10	4079	2
2	B1	sample1-rep2	3500 nt to 5389 nt	11	4091	2.2
3	C1	sample1-rep3	3500 nt to 5389 nt	12	4089	2.4
1	A2	sample2-rep1	3500 nt to 5389 nt	20	4053	4
2	B2	sample2-rep2	3500 nt to 5389 nt	21	4061	4.2
3	C2	sample2-rep3	3500 nt to 5389 nt	22	4065	4.4
1	A3	sample3-rep1	3500 nt to 5389 nt	30	4045	6
2	B3	sample3-rep2	3500 nt to 5389 nt	31	4033	6.2
3	C3	sample3-rep3	3500 nt to 5389 nt	32	4037	6.4
1	A4	sample4-rep1	3500 nt to 5389 nt	40	4089	8
2	B4	sample4-rep2	3500 nt to 5389 nt	41	4069	8.2
3	C4	sample4-rep3	3500 nt to 5389 nt	42	4061	8.4
1	A5	sample5-rep1	3500 nt to 5389 nt	50	4061	10
2	B5	sample5-rep2	3500 nt to 5389 nt	51	4067	10.2
3	C5	sample5-rep3	3500 nt to 5389 nt	52	4070	10.4
1	A6	sample6-rep1	3500 nt to 5389 nt	60	4009	12
2	B6	sample6-rep2	3500 nt to 5389 nt	61	3998	12.2
3	C6	sample6-rep3	3500 nt to 5389 nt	62	4097	12.4
1	A7	sample7-rep1	3500 nt to 5389 nt	70	4071	14
2	B7	sample7-rep2	3500 nt to 5389 nt	71	4049	14.2
3	C7	sample7-rep3	3500 nt to 5389 nt	72	4060	14.4

REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV
1	A1	sample1-rep1	5389 nt to 13000 nt	1	6774	0.2
2	B1	sample1-rep2	5389 nt to 13000 nt	2	5534	0.42
3	C1	sample1-rep3	5389 nt to 13000 nt	3	5684	0.64
1	A2	sample2-rep1	5389 nt to 13000 nt	2	6916	0.4
2	B2	sample2-rep2	5389 nt to 13000 nt	3	4079	0.62
3	C2	sample2-rep3	5389 nt to 13000 nt	4	5530	0.84
1	A3	sample3-rep1	5389 nt to 13000 nt	3	6870	0.6
2	B3	sample3-rep2	5389 nt to 13000 nt	4	6807	0.82
3	C3	sample3-rep3	5389 nt to 13000 nt	5	6551	1.04
1	A4	sample4-rep1	5389 nt to 13000 nt	4	7320	0.8
2	B4	sample4-rep2	5389 nt to 13000 nt	5	7000	1.02
3	C4	sample4-rep3	5389 nt to 13000 nt	6	6970	1.24
1	A5	sample5-rep1	5389 nt to 13000 nt	5	7135	1
2	B5	sample5-rep2	5389 nt to 13000 nt	6	7094	1.22
3	C5	sample5-rep3	5389 nt to 13000 nt	7	6740	1.44
1	A6	sample6-rep1	5389 nt to 13000 nt	6	4079	1.2
2	B6	sample6-rep2	5389 nt to 13000 nt	7	5436	1.42
3	C6	sample6-rep3	5389 nt to 13000 nt	8	8653	1.64
1	A7	sample7-rep1	5389 nt to 13000 nt	7	7717	1.4
2	B7	sample7-rep2	5389 nt to 13000 nt	8	7570	1.62
3	C7	sample7-rep3	5389 nt to 13000 nt	9	8404	1.84

RESULTS FOR VALIDATION DATA											
REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS
							Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	3500 nt to 5389 nt	10	4079	2	sample1-rep1	11.00	1.00	9.09	FAIL
2	B1	sample1-rep2	3500 nt to 5389 nt	11	4091	2.2					
3	C1	sample1-rep3	3500 nt to 5389 nt	12	4089	2.4					
1	A2	sample2-rep1	3500 nt to 5389 nt	20	4053	4	sample2-rep1	21.00	1.00	4.76	FAIL
2	B2	sample2-rep2	3500 nt to 5389 nt	21	4061	4.2					
3	C2	sample2-rep3	3500 nt to 5389 nt	22	4065	4.4					
1	A3	sample3-rep1	3500 nt to 5389 nt	30	4045	6	sample3-rep1	31.00	1.00	3.23	FAIL
2	B3	sample3-rep2	3500 nt to 5389 nt	31	4033	6.2					
3	C3	sample3-rep3	3500 nt to 5389 nt	32	4037	6.4					
1	A4	sample4-rep1	3500 nt to 5389 nt	40	4089	8	sample4-rep1	41.00	1.00	2.44	FAIL
2	B4	sample4-rep2	3500 nt to 5389 nt	41	4069	8.2					
3	C4	sample4-rep3	3500 nt to 5389 nt	42	4061	8.4					
1	A5	sample5-rep1	3500 nt to 5389 nt	50	4061	10	sample5-rep1	51.00	1.00	1.96	FAIL
2	B5	sample5-rep2	3500 nt to 5389 nt	51	4067	10.2					
3	C5	sample5-rep3	3500 nt to 5389 nt	52	4070	10.4					
1	A6	sample6-rep1	3500 nt to 5389 nt	60	4009	12	sample6-rep1	61.00	1.00	1.64	PASS
2	B6	sample6-rep2	3500 nt to 5389 nt	61	3998	12.2					
3	C6	sample6-rep3	3500 nt to 5389 nt	62	4097	12.4					
1	A7	sample7-rep1	3500 nt to 5389 nt	70	4071	14	sample7-rep1	71.00	1.00	1.41	PASS
2	B7	sample7-rep2	3500 nt to 5389 nt	71	4049	14.2					
3	C7	sample7-rep3	3500 nt to 5389 nt	72	4060	14.4					

REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
							Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	5389 nt to 13000 nt	1	6774	0.2	sample1-rep1	2.00	1.00	50.00	
2	B1	sample1-rep2	5389 nt to 13000 nt	2	5534	0.42					
3	C1	sample1-rep3	5389 nt to 13000 nt	3	5684	0.64					
1	A2	sample2-rep1	5389 nt to 13000 nt	2	6916	0.4	sample2-rep1	3.00	1.00	33.33	
2	B2	sample2-rep2	5389 nt to 13000 nt	3	4079	0.62					
3	C2	sample2-rep3	5389 nt to 13000 nt	4	5530	0.84					
1	A3	sample3-rep1	5389 nt to 13000 nt	3	6870	0.6	sample3-rep1	4.00	1.00	25.00	
2	B3	sample3-rep2	5389 nt to 13000 nt	4	6807	0.82					
3	C3	sample3-rep3	5389 nt to 13000 nt	5	6551	1.04					
1	A4	sample4-rep1	5389 nt to 13000 nt	4	7320	0.8	sample4-rep1	5.00	1.00	20.00	
2	B4	sample4-rep2	5389 nt to 13000 nt	5	7000	1.02					
3	C4	sample4-rep3	5389 nt to 13000 nt	6	6970	1.24					
1	A5	sample5-rep1	5389 nt to 13000 nt	5	7135	1	sample5-rep1	6.00	1.00	16.67	
2	B5	sample5-rep2	5389 nt to 13000 nt	6	7094	1.22					
3	C5	sample5-rep3	5389 nt to 13000 nt	7	6740	1.44					
1	A6	sample6-rep1	5389 nt to 13000 nt	6	4079	1.2	sample6-rep1	7.00	1.00	14.29	
2	B6	sample6-rep2	5389 nt to 13000 nt	7	5436	1.42					
3	C6	sample6-rep3	5389 nt to 13000 nt	8	8653	1.64					
1	A7	sample7-rep1	5389 nt to 13000 nt	7	7717	1.4	sample7-rep1	8.00	1.00	12.50	
2	B7	sample7-rep2	5389 nt to 13000 nt	8	7570	1.62					
3	C7	sample7-rep3	5389 nt to 13000 nt	9	8404	1.84					

## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2023 03 17\14-23-19\2023 03 17 14H 23M.raw  
**Created:** Friday, March 17, 2023 2:49:06 PM  
**Number of capillaries:** 11  
**Array serial number:** 031221-22SFS  
**Effect length:** 33 cm  
**Array usage count:** 16  
**Instrument type:** 5300 Fragment Analyzer  
**Instrument controller software version:** 3.1.0.12  
**Device serial number:** MY2105AB19

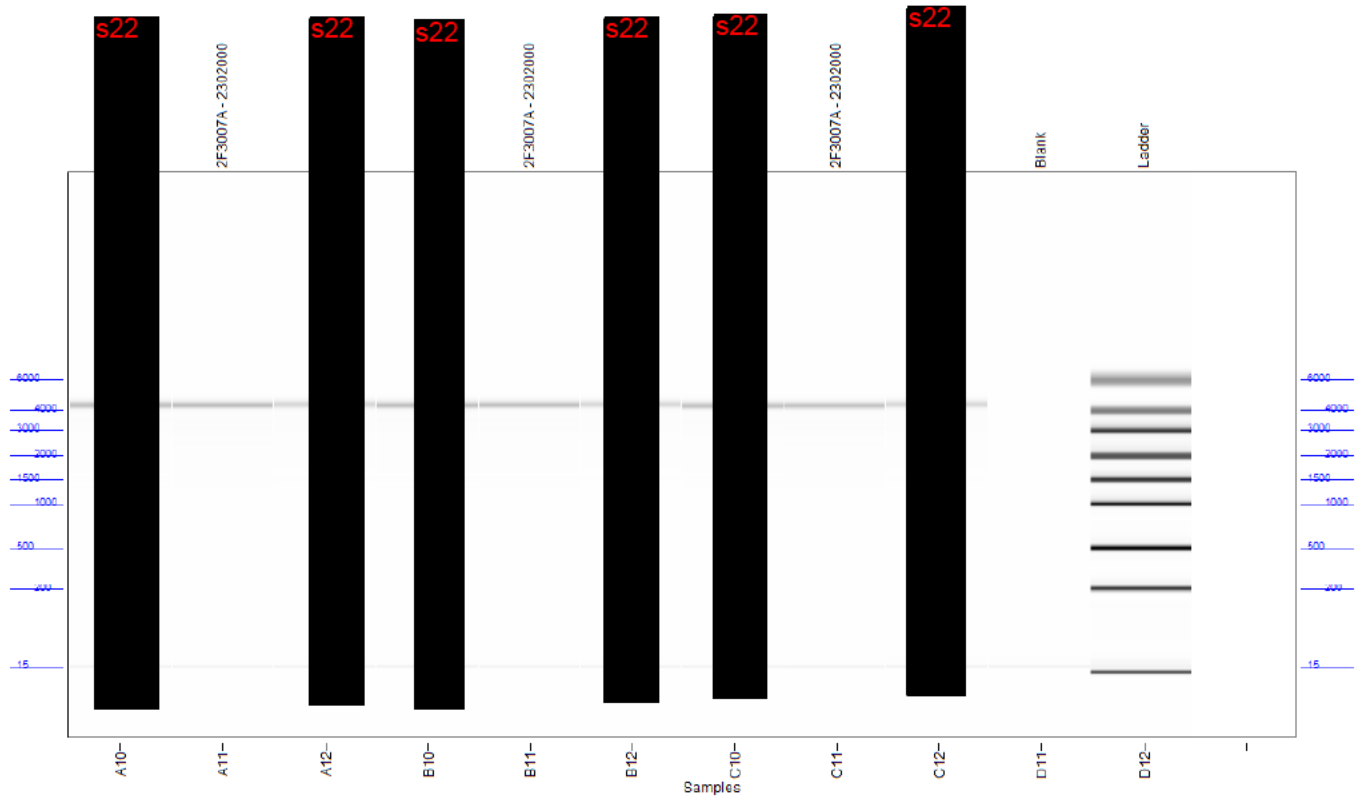
### Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds  
**Gel prime:** No  
**Full conditioning:** Yes  
**Gel prime to buffer:** Yes  
**Gel selection:** Gel 2  
**Perform prerun:** 8.0 kV, 30 sec.  
**Rinse:** No  
**Marker 1:** No  
**Rinse:** Tray: 3, Row: A, Dip count: 2  
**Sample injection:** 5.0 kV, 15 sec.  
**Separation:** 8.0 kV, 60.0 min.  
**Tray name:** Tray-1

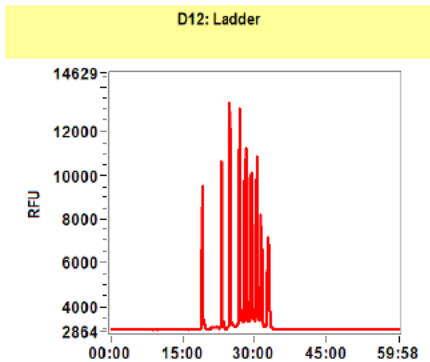
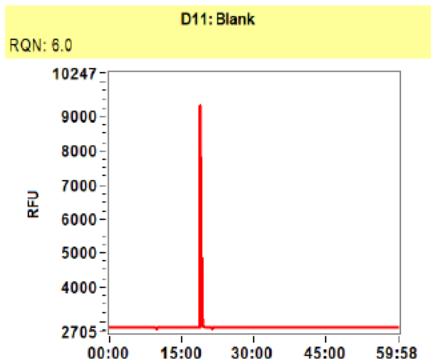
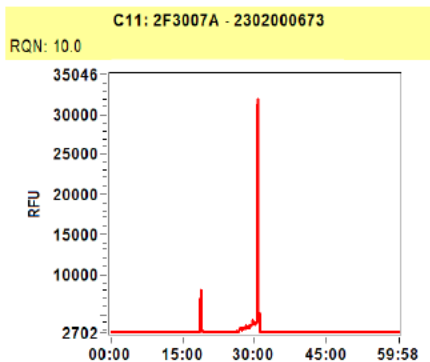
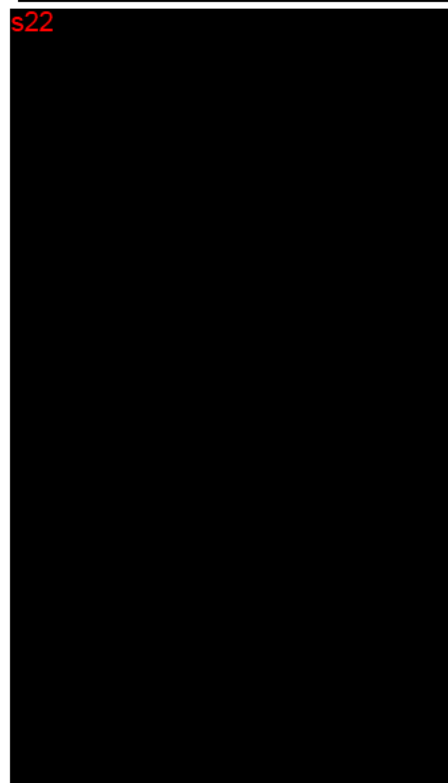
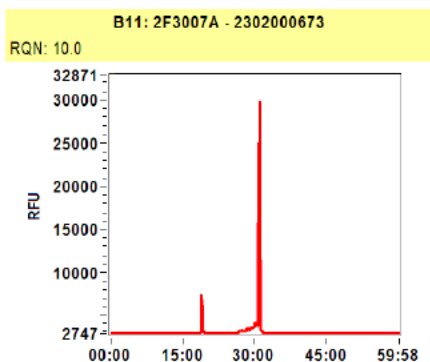
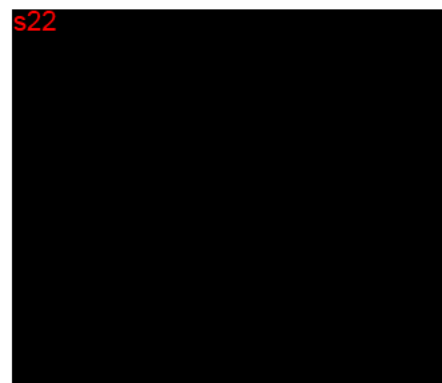
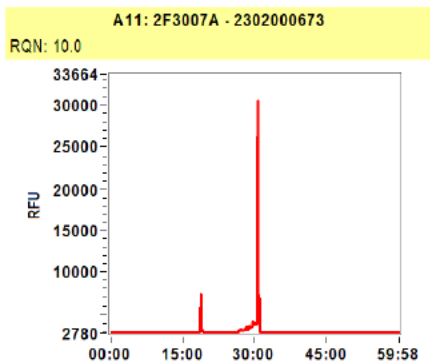
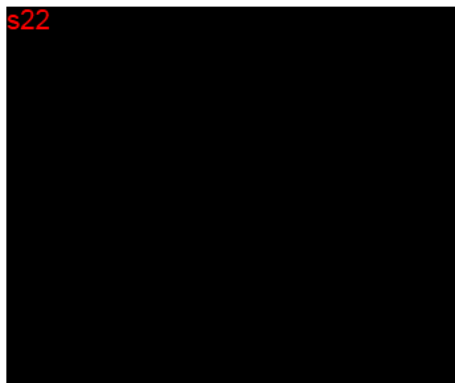
**Analysis mode:** RNA (Eukaryotic)

### Notes

### Gel Image



Filename and data path: C:\Agilent Technologies\Data\2023 03 17\14-23-19\2023 03 17 14H 23M.raw

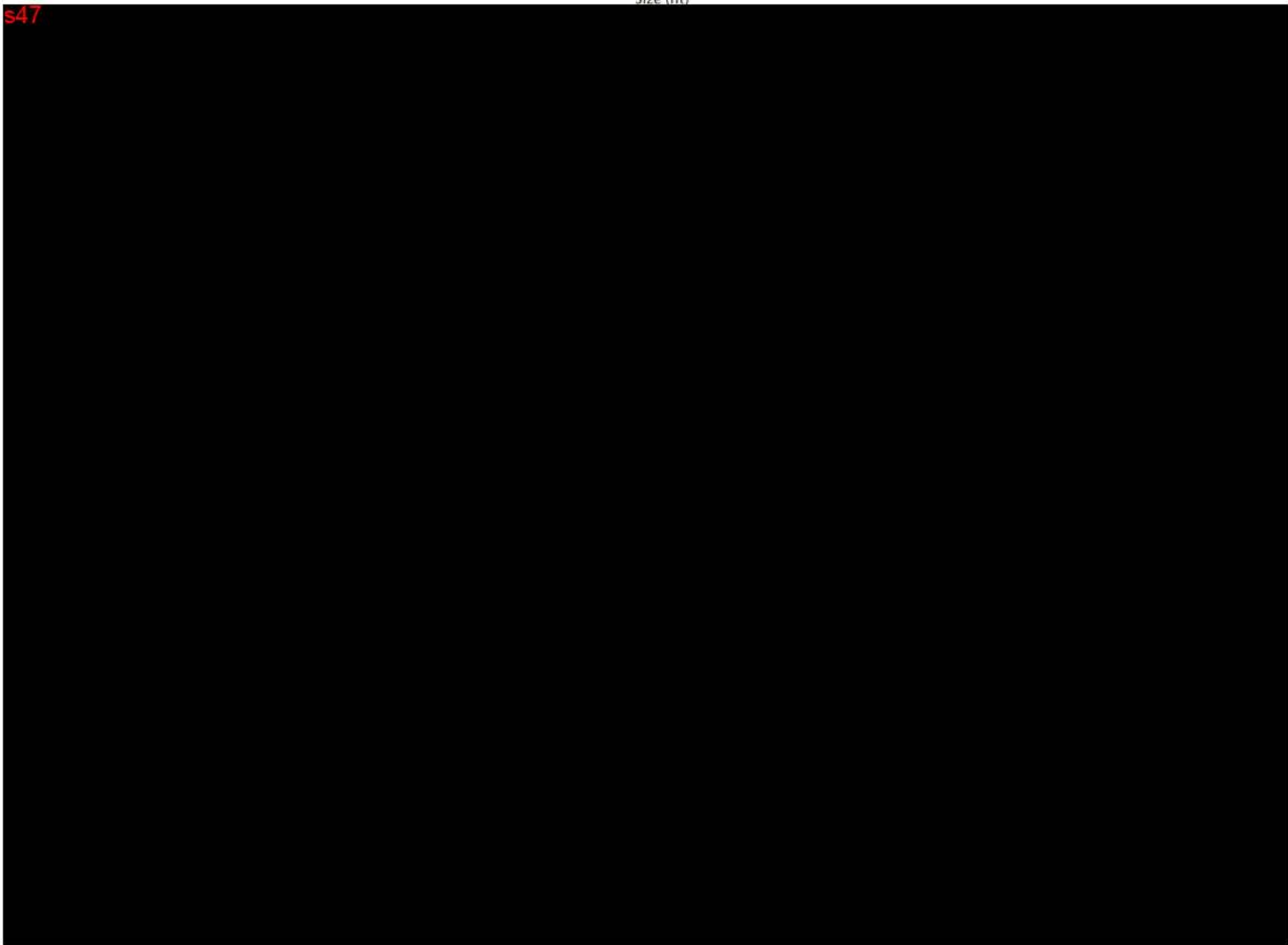
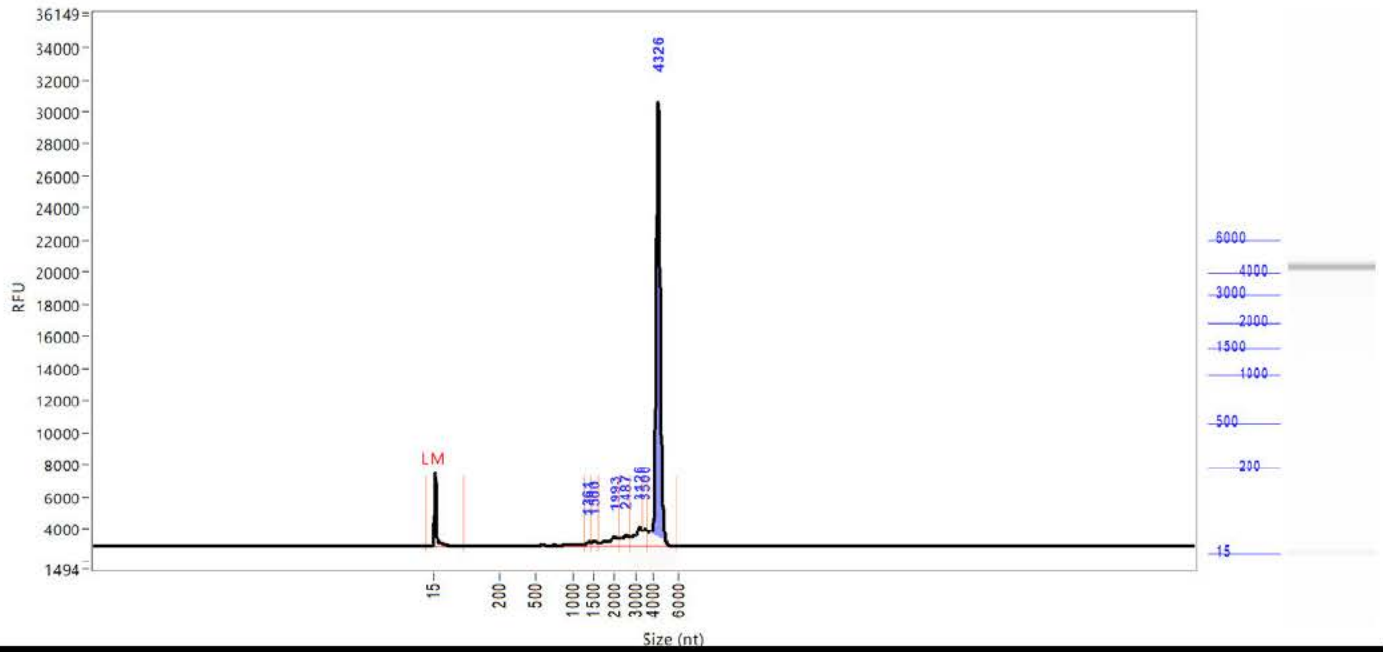
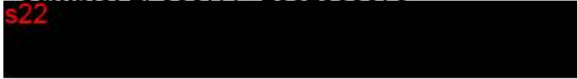




s22



Sample: 2F3007A - 2302000673



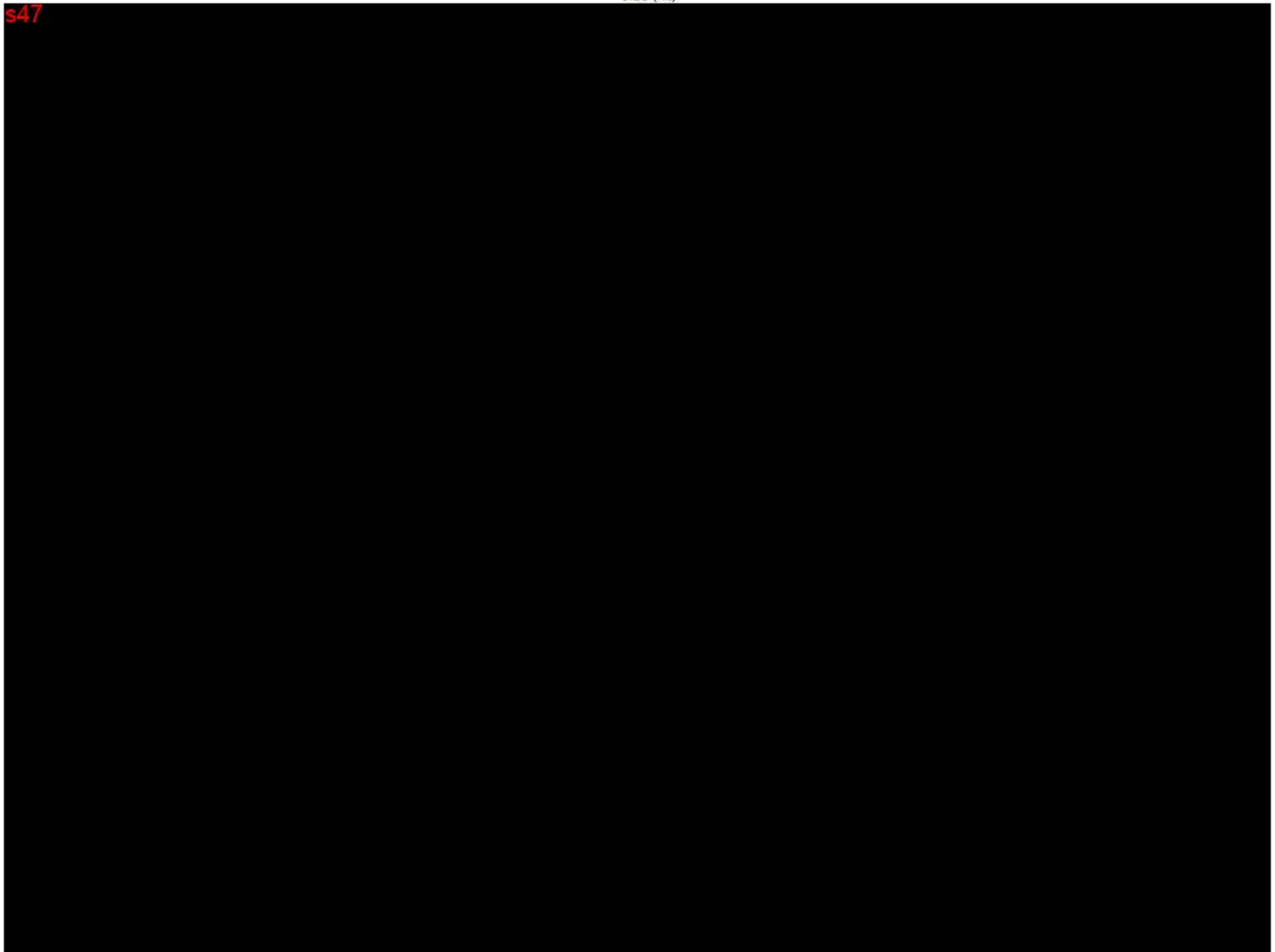
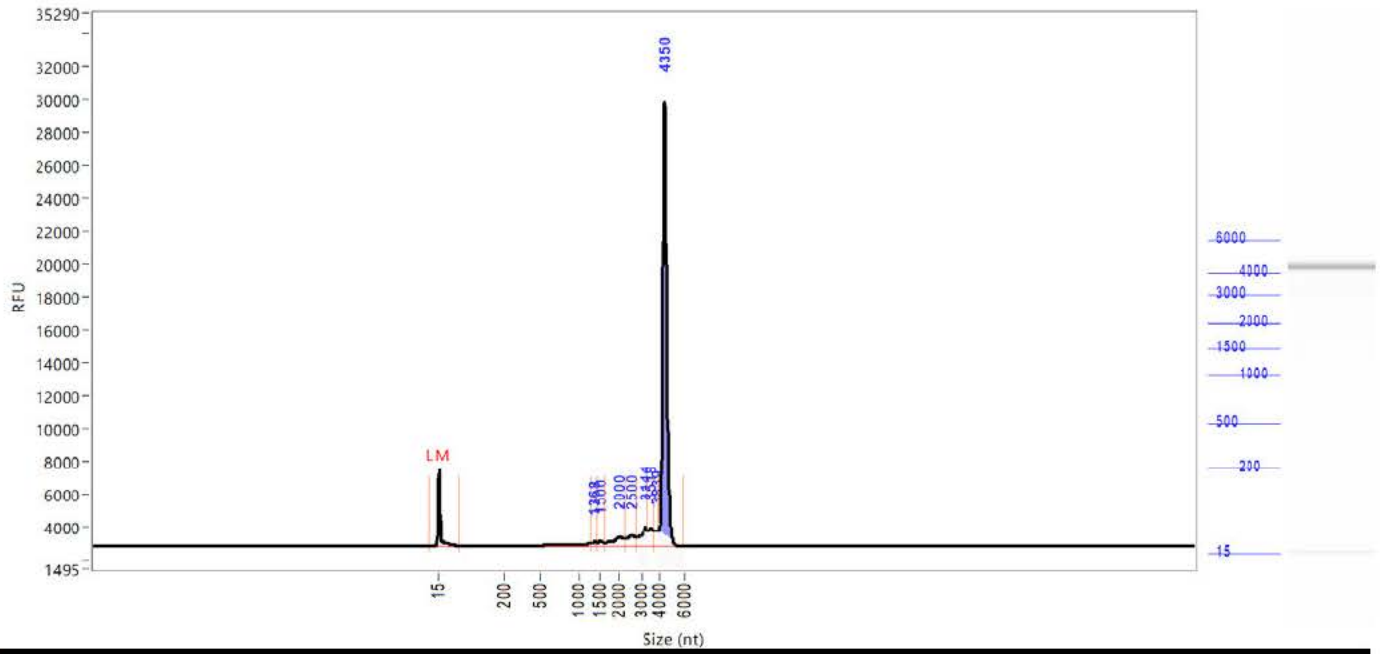
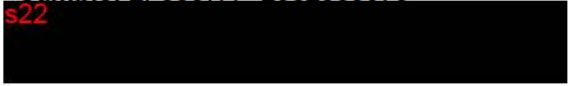
S22



s22



Sample: 2F3007A - 2302000673



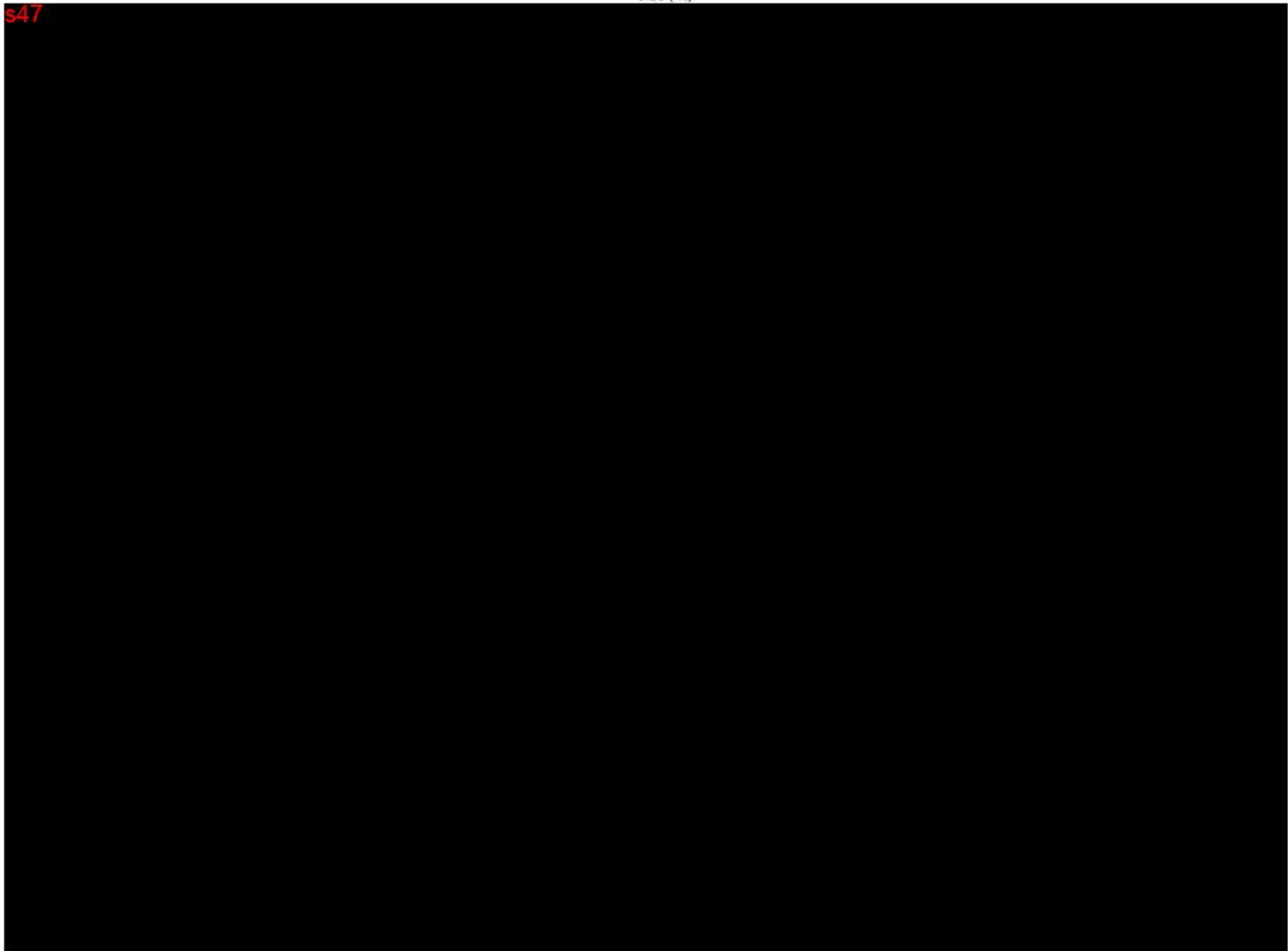
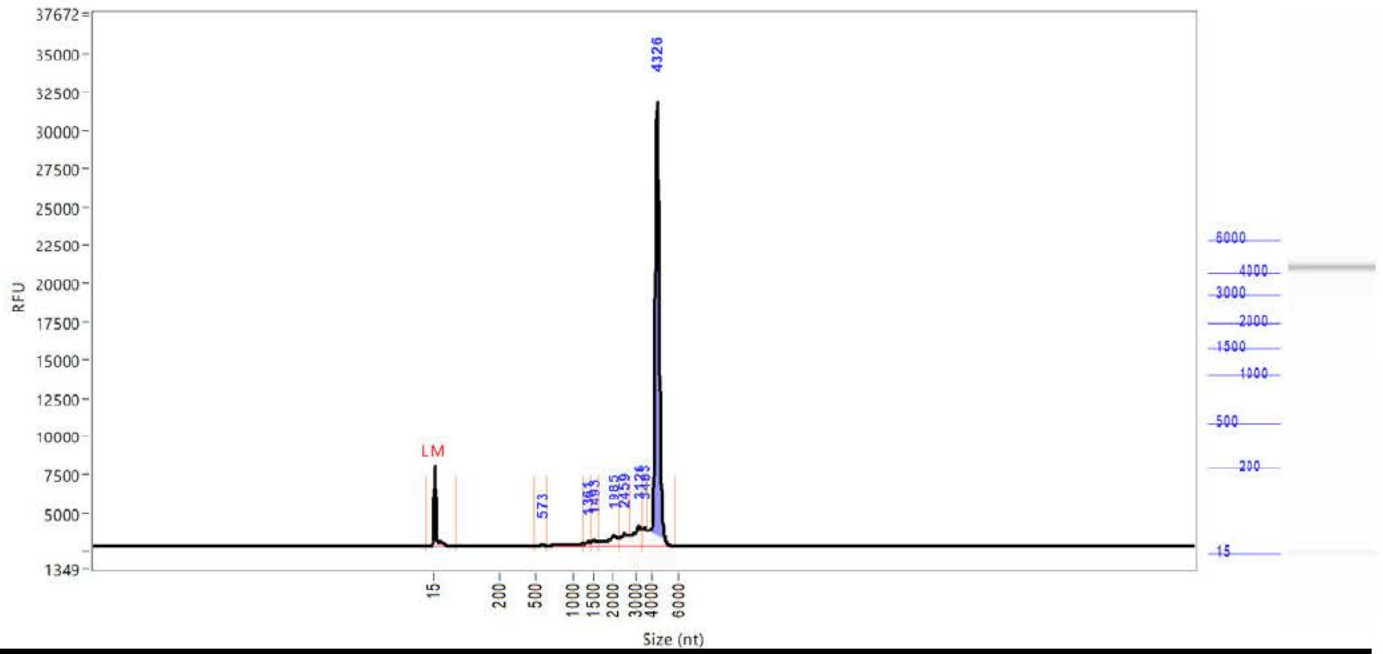
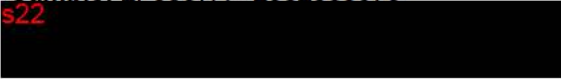
S22



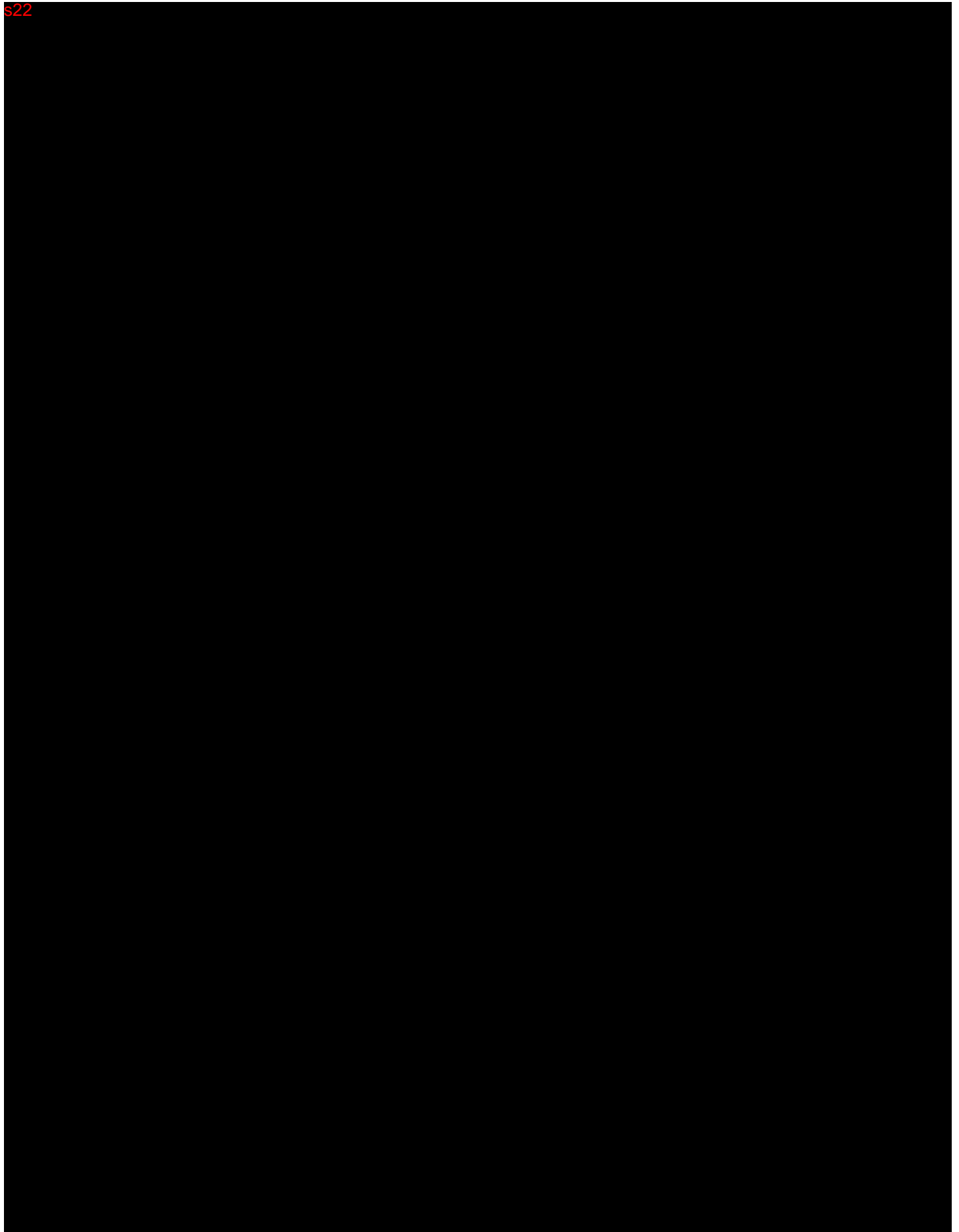
s22



Sample: 2F3007A - 2302000673

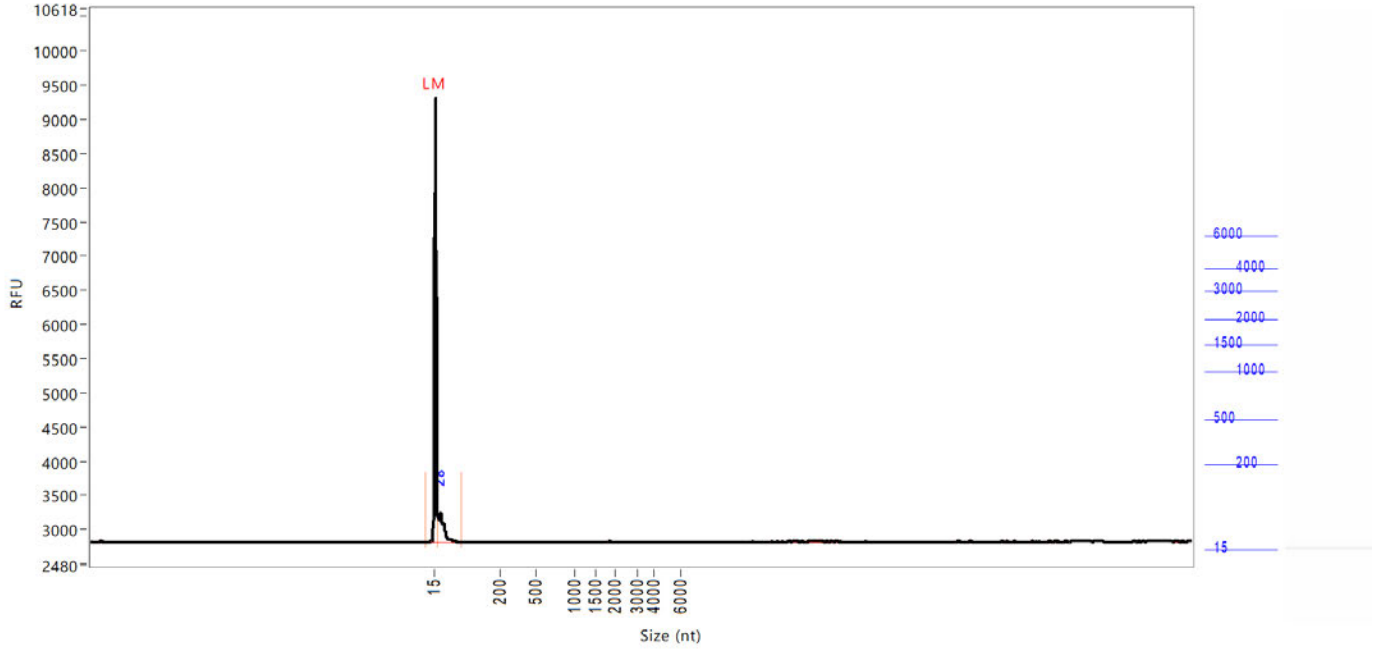






s22

**Sample:** Blank  
**Well location:** D11  
**Created:** Friday, March 17, 2023 2:49:06 PM

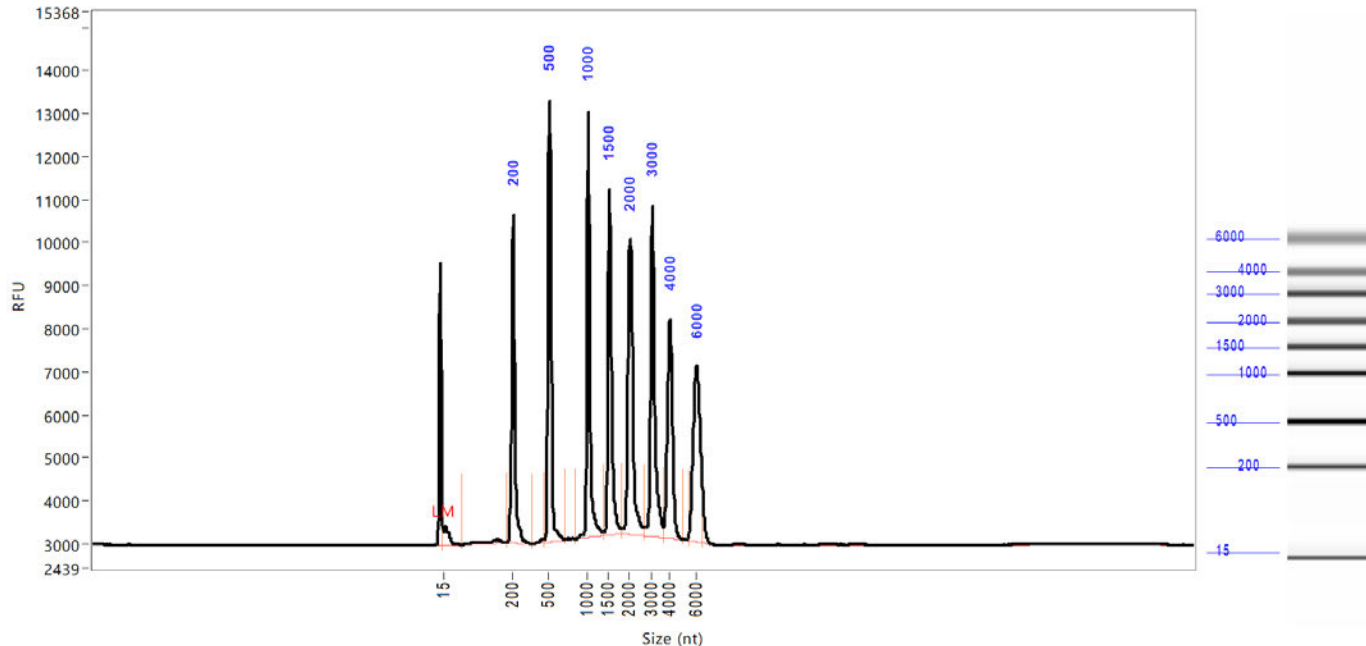


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.1151	0	23	6504
2	28	0.2503	23	90	426

TIC: 0.2503 ng/uL  
 TIM: 22.0050 nmole/L  
 Total concentration: 0.2663 ng/uL  
 28s/18s: 0.0  
 RQN 6.0

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: Y    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Friday, March 17, 2023 2:49:06 PM



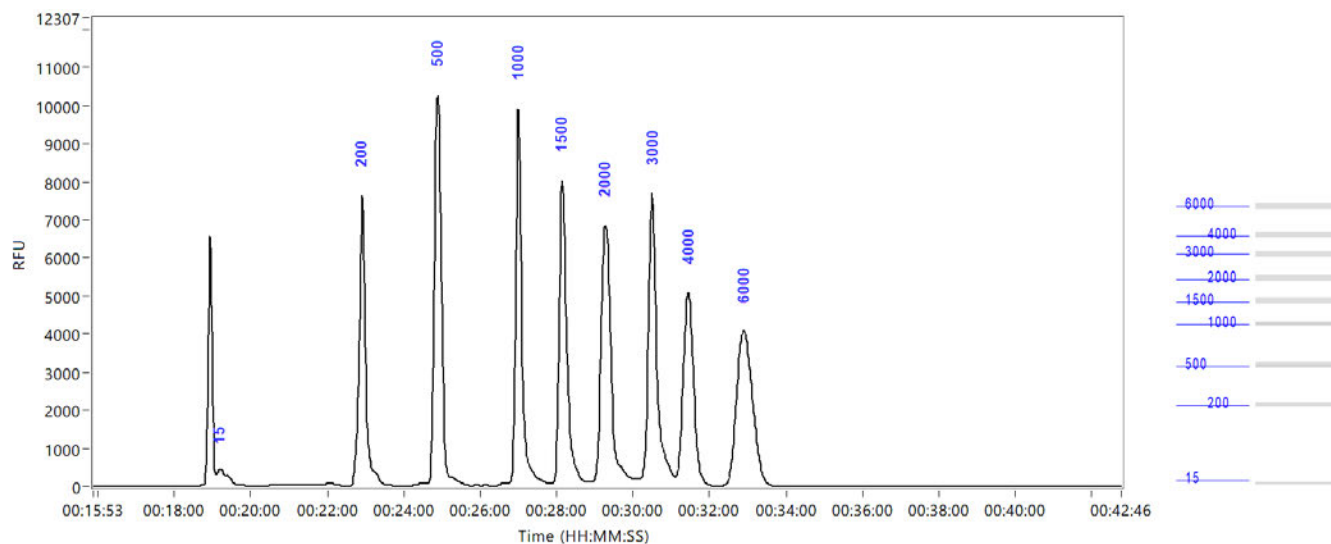
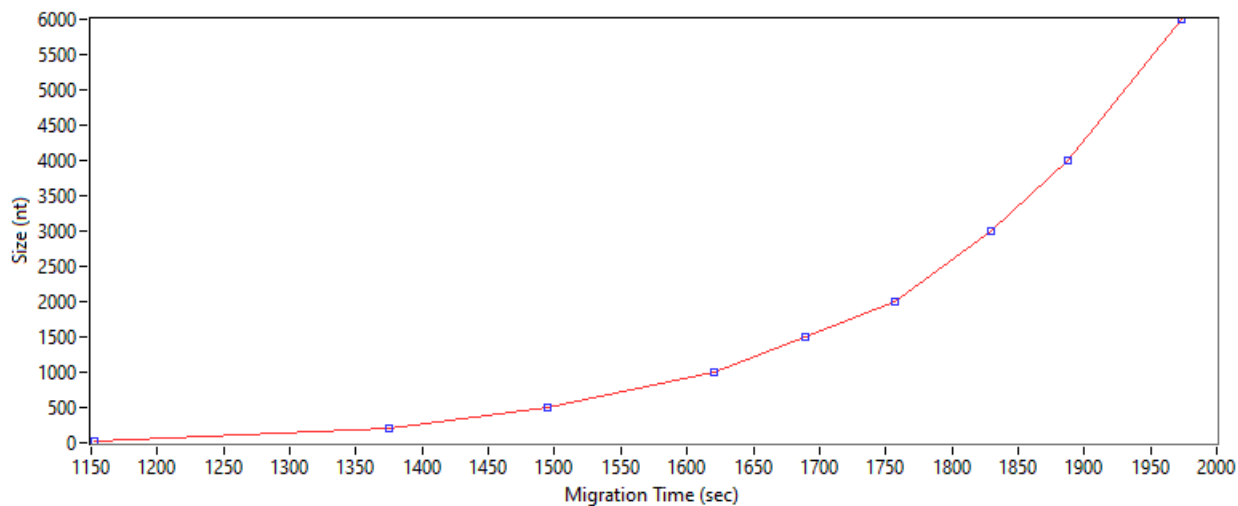
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.0215	9	61	445
2	200	2.1437	182	361	7636
3	500	3.0316	457	701	10254
4	1000	2.3052	832	1361	9888
5	1500	2.2248	1361	1799	8008
6	2000	2.4533	1799	2667	6853
7	3000	2.2089	2667	3625	7689
8	4000	1.7346	3625	5047	5085
9	6000	1.8112	5489	6414	4084

TIC: 17.9133 ng/uL  
 TIM: 71.3012 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6      Sample min peak height: 200      Sample baseline V to V?: Y      Sample baseline V to V points: 3  
 Sample filter: Binomial      Number of points for filter: 9      Sample start region (min): 0      Sample end region (min): 60  
 Marker peak width (sec): 6      Marker min peak height: 100      Marker baseline V to V?: Y      Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU      Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder      Final concentration (ng/uL): 8.0000      Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Friday, March 17, 2023 2:49:06 PM  
**Fit type:** Point to point

Calibration curve





<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-18
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b>	<b>Review:</b> 21/04/2023
<b>Title:</b> Fragment Analyzer – Worksheet - General	

### Worksheet for Fragment Analyzer

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-31	<b>Analyst</b>	s22
<b>TRIM link to data files</b>	Enter text.	<b>Test Date</b>	17/03/2023
<b>Modifications to SOP</b>	Using a thermomixer instead of thermocycler: <u>D21-3185919</u>		

Pipettes & Equipment	
Name	LIMS#
P20	33054
P200	33438
P1000	33440
Thermomixer	33475

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	Enter text.	MY21080527	Enter a date.
Inlet Buffer	DNF-355-0300	6682787	6/05/2023
Rinse Buffer	DNF-497-0125	6678610	18/04/2023
Capillary Storage Buffer	GP-440-0100	6611501	19/05/2023
Capillary conditioning solution	DNF-475-0100	6675084	29/03/2023
RNA Separation Gel	DNF-265-0500	6682983	8/06/2023
Intercalating dye	DNF-600-U030	6691202	24/06/2023
Blank	DNF-300-0008	6681489	2/05/2023
RNA Ladder	DNF-382-U020	6692232	1/07/2023
Diluent Marker	DNF-369-0004	6622872	12/07/2023
DEPC water	In house	20230131/01	30/01/2024
20% T-X100 / 30% EtOH solution	In house	ST17Mar23-1	17/06/2023

GP-4400100

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT ( <b>Drawer B</b> )	17/03/2023	18/03/2023  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT ( <b>Drawer M</b> )	17/03/2023	18/03/2023  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT ( <b>Drawer 3</b> )	8/03/2023	22/03/2023  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	17/03/2023	31/03/2023  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	17/03/2023	19/03/2023  48 hours
<p><b>Empty waste tray and waste bottle</b></p> <p>Reagents can be scaled up if required – this table provides the minimum for a single run. Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use</p> <p>Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use</p> <p>Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.</p>			

## 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S11a	S10a	S9a	S8a	S7a	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	S11b	S10b	S9b	S8b	S7b	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	S11c	S10c	S9c	S8c	S7c	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	S12a	S12b	S12c	S13a	S13b	S13c	S14a	S14b	S14c	BF-25	BF-25	L

**S1-14** = Samples in triplicate (a, b or c), note this worksheet only contains enough fields for 6 samples.

**RM** = Reference material

**BF-25** = Blank solution provided in kit,

**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test.

System Suitability Criteria					
Tested	Well	Parameter	Limits	Results	PASS/FAIL
RNA Ladder	D12	Profile of RNA Ladder	Must be visually comparable to example given in SOP	Comply	PASS
RNA Ladder	D12	All ladder peaks must be present	15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000	Comply	PASS
RNA Ladder	D12	Peak heights for individual RNA ladder peaks	< 60,000 RFU	Comply	PASS
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.

Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
-------------	-----------------	-------------	-------------	-------------	-----------------



Assay Acceptance Criteria					
Tested RM (LIMS), blank, ladder?	Well	Parameter	Limits	Results	PASS/FAIL
RM LIMS 2111004298	A12 B12 C12	Profile	Must be visually comparable to SOP	Comply	PASS
RM LIMS 2111004298	A12 B12 C12	Lower marker peak	Must be present	Comply	PASS
RM LIMS 2111004298	A12 B12 C12	Significant negative peaks or baseline drifts	Not present	Comply	PASS
RM LIMS 2111004298	A12 B12 C12	Peak heights	Must be between 5000-60000 for 2/3 replicates	Comply	PASS
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Dilutions / Calculation / Notes					
Working stock 1: 100 ng/uL = 20 x 470 ng/uL + 74 uL DEPC					
Working stock 2: 33 ng/uL = 20 x 100 ng/uL + 40 uL Triton/ethanol mix					

Sample 1					
Plate location (wells)	A10 B10 C10				
LIMS #	2302000613				
BATCH #	s22				
EXPIRY	31/08/2023				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Peak height of main peak	5000-60000 RFU	Between 27000-29044			PASS
Lower Marker peak	Must be present	Comply			PASS
Negative peaks or baseline drift	Must not be present	Comply			PASS
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Main peak	s47	s22			
LMS	s22				
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
PASS					
Analysist			s22		
Checked by			s22		
Sample Dilutions / Calculation / Notes					
Sample preparation 33 ng/uL = 20ul x 100 ng/uL + 40 uL Triton/ethanol mix					

Sample 2					
Plate location (wells)	A11 B11 C11				
LIMS #	2302000673				
BATCH #	2F3007A				
EXPIRY	31/08/2023				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Peak height of main peak	5000-60000 RFU	Between 25022-27159			PASS
Lower Marker peak	Must be present	Comply			PASS
Negative peaks or baseline drift	Must not be present	Comply			PASS
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Main peak	s47	s47			PASS
LMS	N/A				PASS
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
PASS					
Analysist			s22		
Checked by			s22		
Sample Dilutions / Calculation / Notes					
Sample preparation 33 ng/uL = 20ul x 100 ng/uL + 40 uL Triton/ethanol mix					

Sample 3					
Plate location (wells)	Choose an item.				
LIMS #	Click or tap here to enter text.				
BATCH #	Click or tap here to enter text.				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
Choose an item.					
Analysist			Enter text.		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
Enter text.					

Sample 4					
Plate location (wells)	Choose an item.				
LIMS #	Click or tap here to enter text.				
BATCH #	Click or tap here to enter text.				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
Choose an item.					
Analysist			Enter text.		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
Enter text.					

Sample 5					
Plate location (wells)	Choose an item.				
LIMS #	Click or tap here to enter text.				
BATCH #	Click or tap here to enter text.				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
Choose an item.					
Analysist			Enter text.		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
Enter text.					

Sample 6					
Plate location (wells)	Choose an item.				
LIMS #	Click or tap here to enter text.				
BATCH #	Click or tap here to enter text.				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results	Comments		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Enter text.	Enter text.	Enter text.	Choose an item.		
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
Choose an item.					
Analysist			Enter text.		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
Enter text.					

**Notes**

Enter text.





Australian Government

Department of Health and Aged Care

Therapeutic Goods Administration

Laboratories Branch

<b>Type:</b> Biotherapeutics\BEE\Forms	<b>Number:</b> Bio-BEE-Form-39 / <b>Version:</b> 3
<b>Owner:</b> s22	<b>Approver:</b> s22
<b>Active:</b> 10/05/2022	<b>Review:</b> 10/11/2023
<b>Title:</b> Endotoxin Routine Assay Worksheet	

## Endotoxin Routine Assay Worksheet

Assay ID: 15Mar2023 Operator: s22

### Limulus Amoebocyte Lysate (LAL)

Lysate batch and expiry recorded on software for each assay

Ensure sensitivity of LAL batch has been confirmed. 'Lysate preparation details' shown below

### Recombinant Factor C (rFC)

rFC Enzyme, Fluorogenic Substrate & rFC Assay Buffer batches and expiry dates recorded on software for each assay

Ensure sensitivity of the rFC batch has been confirmed. 'rFC Reagent preparation details' shown below

### Control Standard Endotoxin (CSE) – refer to Bio-BEE-Method 5 and Bio-BEE-Form 37

CSE batch and expiry recorded on software for each assay

Reconstitution details for either KLAL or rFC – see Trim File D23-5206129

CSE Lot Number: 0001028884 Conc 50 EU/mL

### LAL Reagent Water (LRW) Lot Number: 0000981166 Expiry: 16Sep2022

How many samples were linked to this assay? 2

**This form is used for recording the assay details and results and only gives the method in point form. See Bio-BEE-SOP 28 and appropriate method for the detailed procedure.**

To avoid endotoxin contamination, use careful technique and **pyrogen free** equipment.

### Preparation of Assay

- Fill out the appropriate forms from the Quality Management System (QMS)
- Retrieve the required kit reagents from cold storage to equilibrate to room temperature
- Turn on plate reader and computer and follow the steps as detailed in Bio-BEE-Method 4

### Preparation of CSE (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare CSE as detailed in Bio-BEE-Method 5. CSE dilutions can be dispensed to the plate as they are prepared to save mixing time

- CSE is set up as in the table below - record %CV results from the final report

#### For a KLAL (KQCL) assay

Concentration	Plate wells ID	% CV
50 EU/ml	F1 – F2	0.74
5 EU/ml	E1 – E2	0.31
0.5 EU/ml	D1 – D2	0.19
0.05 EU/ml	C1 – C2	0.82
0.005 EU/ml	B1 – B2	0.40
Blank	A1 – A2	

#### For an rFC assay

Concentration	Plate wells ID	% CV
5 EU/ml	E1 – E2	-
0.5 EU/ml	D1 – D2	-
0.05 EU/ml	C1 – C2	-
0.005 EU/ml	B1 – B2	-
Blank	A1 – A2	

- Dispense 100 µl of the appropriate dilution of CSE into the appropriate wells of the plate.
- Continue with procedure as per the appropriate method

#### Preparation of Samples (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare sample dilutions as in Bio-BEE-Form 42
- Pipette 10 µl of the 5 EU/ml standard to the appropriate PPC wells as per the plate layout
- Dispense 100 µl of the final sample dilutions into the 4 appropriate wells as per the plate layout

#### Starting the Assay

- The plate is then ready for the reaction. Prepare the software as set out in the Bio-BEE-Method 4
- “Run” the Template prepared earlier. Follow the prompts to the Pre-warming step

#### If performing a KLAL assay

- Prepare the required lysate vial/s (Bop-BEE-Method 6) and pour into the reagent reservoir

Lysate Preparations Details -

Lysate Lot Number	YL002L2U24	Expiry 11Jul2024
Date sensitivity confirmed	09Feb2023	E23-509118
Reconstitute lysate with	2.6 (3x867 uL)	mL of LAL Reagent Water (LRW)
Date reconstituted	15Mar2023	-
Operator(s)	S22	-
Use by date	29Mar2023	(Lonza KQCL- 14 days from reconstitution, at below -10°C)

**OR**

*If performing an rFC assay*

- Make up the required volume of rFC reagent directly into the reservoir (Bio-BEE-Method 6)

rFC Reagent Preparations Details – (Note: once prepared, working reagent cannot be stored)

	Lot	Expiry	Volume
Date sensitivity confirmed	-	-	
Fluorogenic Substrate	-	-	- µL
rFC Assay Buffer	-	-	- µL
rFC Enzyme Solution	-	-	- µL

- Open cover – if using the Spectramax use the software to open and close the drawer
- Add 100 µl of either lysate (KLAL) **OR** working reagent (rFC) to each of the assay wells, carefully, and as quickly as possible
- Close the drawer on the plate reader and click OK to start the run. **Do not open drawer**

**Acceptance Criteria – for KLAL**

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>-0.998</u>
Slope between -0.400 and -0.100	<u>-0.237</u>
Y intercept between 2.500 and 3.500	<u>3.132</u>
Mean reaction times of blank $\geq$ mean reaction times of lowest standard	<u>Yes</u>
Coefficient of variation (CV) values for all standards are $< 10\%$	<u>Yes</u>
Were all acceptance criteria for the standard curve met?	<u>Yes</u>

## Acceptance Criteria – for *rFC*

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	=
Slope between 0.760 and 1.110	=
Y intercept between 2.500 and 5.000	=
Mean RFU of blank $\leq$ mean RFU of lowest standard	=
Coefficient of variation (CV) values for all standards are $< 25\%$	=
Were all acceptance criteria for the standard curve met?	=

## Conclusions

Follow procedures for 'Recording Results' detailed in Bio-BEE-Method 7

## Notes

Click or tap here to enter text.

## Data from Smear Analysis Table

1. Ensure to export smear data for the main peak and for the LMS for ALL wells in rows ABC&D of the assay plate
2. Enter the smear data into the table below. If the SOP plate layout was strictly followed, the calculations tab will show automatically calculated average, standard deviation and %CV (%RSD) for the two smear sets.
3. Pass/Fail will be automatically determined using the parameters entered in the Calculations tab.

Well #	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV
A1	A1	Blank	3700 nt to 4800 nt	0.0053	5.6	0.0042	3964	2.41
A1	A1	Blank	4800 nt to 13000 nt	0.0004	0.4	0.0001	9438	2.84
A2	A2	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A2	A2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A3	A3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A3	A3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A4	A4	Blank	3700 nt to 4800 nt	0.0001	0.3	0.0001	3716	0.23
A4	A4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A5	A5	Blank	3700 nt to 4800 nt	0.0021	1.4	0.0017	3799	1.44
A5	A5	Blank	4800 nt to 13000 nt	0.0001	0	0	7561	0.15
A6	A6	Blank	3700 nt to 4800 nt	0.0076	2.3	0.0061	3846	2.44
A6	A6	Blank	4800 nt to 13000 nt	0.0007	0.2	0.0003	7872	3.87
A7	A7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
A7	A7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A8	A8	Blank	3700 nt to 4800 nt	0.0002	1.8	0.0002	3897	1.42
A8	A8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A9	A9	Blank	3700 nt to 4800 nt	0.0064	15.4	0.0048	4164	2.01
A9	A9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A10	A10	Blank	3700 nt to 4800 nt	0.0256	45.8	0.0197	4044	2.18
A10	A10	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
A11	A11	GM6363-2301000423						
A11	A11	GM6363-2301000423						
B1	B1	Blank	3700 nt to 4800 nt	0.0151	84.6	0.0112	4219	2.32
B1	B1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B2	B2	Blank	3700 nt to 4800 nt	0.0014	5.2	0.0011	4164	1.31
B2	B2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B3	B3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B3	B3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B4	B4	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B4	B4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B5	B5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B5	B5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B6	B6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
B6	B6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B7	B7	Blank	3700 nt to 4800 nt	0.0012	1.7	0.0009	4432	1
B7	B7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B8	B8	Blank	3700 nt to 4800 nt	0.0022	3.5	0.0016	4240	2.93
B8	B8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B9	B9	Blank	3700 nt to 4800 nt	0.0007	5	0.0006	3959	6.81
B9	B9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B10	B10	Blank	3700 nt to 4800 nt	0.0246	53.8	0.0186	4120	2.94
B10	B10	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
B11	B11	GM6363-2301000423						
B11	B11	GM6363-2301000423						
C1	C1	Blank	3700 nt to 4800 nt	0.0114	68.2	0.0081	4395	2.96
C1	C1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C2	C2	Blank	3700 nt to 4800 nt	0.0005	3.4	0.0004	4261	0.83
C2	C2	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C3	C3	Blank	3700 nt to 4800 nt	0.0057	8.3	0.0041	4322	4.23
C3	C3	Blank	4800 nt to 13000 nt	0	0	0	9099	0.12
C4	C4	Blank	3700 nt to 4800 nt	0.0009	5.8	0.0007	4274	1.37
C4	C4	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C5	C5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C5	C5	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C6	C6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C6	C6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C7	C7	Blank	3700 nt to 4800 nt	0.0026	39.4	0.0021	3929	1.54
C7	C7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C8	C8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C8	C8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C9	C9	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
C9	C9	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C10	C10	Blank	3700 nt to 4800 nt	0.0018	17.4	0.0014	4081	1.24
C10	C10	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
C11	C11	GM6363-2301000423						
C11	C11	GM6363-2301000423						
D1	D1	Blank	3700 nt to 4800 nt	0.0071	63.3	0.0054	4107	2.03
D1	D1	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	3700 nt to 4800 nt	0.0033	9.9	0.0026	3967	2.56
D2	D2	Blank	4800 nt to 13000 nt	0.0022	6.6	0.0012	5630	14.23
D3	D3	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	3700 nt to 4800 nt	0.0038	7.7	0.003	3968	5.43
D4	D4	Blank	4800 nt to 13000 nt	0.0028	5.8	0.0015	5731	29.36
D5	D5	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	4800 nt to 13000 nt	0.0001	2	0	10686	0.15
D6	D6	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D6	D6	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D9	D9	Blank	3700 nt to 4800 nt	0.0019	6.6	0.0016	3869	4.79
D9	D9	Blank	4800 nt to 13000 nt	0.0006	2	0.0002	9175	11.41
D10	D10	Blank	3700 nt to 4800 nt	0.0002	0.1	0.0002	3795	3.39
D10	D10	Blank	4800 nt to 13000 nt	0.0086	5.3	0.0028	9521	6.83
D11	D11	Blank	3700 nt to 4800 nt	0	0	NaN	NaN	NaN
D11	D11	Blank	4800 nt to 13000 nt	0	0	NaN	NaN	NaN
D12	D12	Ladder						

OFFICIAL

Q-Pulse Document Number	Bio-BPC-Form-13
Title	Fragment Analyzer Spreadsheet - 2 smears
Author	\$22
Owner (Reviewer)	\$22
Approver (Authoriser)	\$22
Date Authorised	23/09/2022
Revision Number	2

Analyst	\$22
Assay Date	16/02/2023

Product specific parameters for Pass/Fail		
minimum	cut off	maximum
\$47		
result >>	\$47	

							% INTEGRITY SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	Sample ID	Average	stdev	%CV	
1	A1	Blank	3700 nt to 4800 nt	5.6	3964	2.41	Blank	52.80	41.69	78.96	FAIL
2	B1	Blank	3700 nt to 4800 nt	84.6	4219	2.32					
3	C1	Blank	3700 nt to 4800 nt	68.2	4395	2.96					
1	A2	Blank	3700 nt to 4800 nt	0	NaN	NaN	Blank	2.87	2.64	92.12	FAIL
2	B2	Blank	3700 nt to 4800 nt	5.2	4164	1.31					
3	C2	Blank	3700 nt to 4800 nt	3.4	4261	0.83					
1	A3	Blank	3700 nt to 4800 nt	0	NaN	NaN	Blank	2.77	4.79	173.21	FAIL
2	B3	Blank	3700 nt to 4800 nt	0	NaN	NaN					
3	C3	Blank	3700 nt to 4800 nt	8.3	4322	4.23					
1	A4	Blank	3700 nt to 4800 nt	0.3	3716	0.23	Blank	2.03	3.27	160.60	FAIL
2	B4	Blank	3700 nt to 4800 nt	0	NaN	NaN					
3	C4	Blank	3700 nt to 4800 nt	5.8	4274	1.37					
1	A5	Blank	3700 nt to 4800 nt	1.4	3799	1.44	Blank	0.47	0.81	173.21	FAIL
2	B5	Blank	3700 nt to 4800 nt	0	NaN	NaN					
3	C5	Blank	3700 nt to 4800 nt	0	NaN	NaN					
1	A6	Blank	3700 nt to 4800 nt	2.3	3846	2.44	Blank	0.77	1.33	173.21	FAIL
2	B6	Blank	3700 nt to 4800 nt	0	NaN	NaN					
3	C6	Blank	3700 nt to 4800 nt	0	NaN	NaN					
1	A7	Blank	3700 nt to 4800 nt	0	NaN	NaN	Blank	13.70	22.27	162.58	FAIL
2	B7	Blank	3700 nt to 4800 nt	1.7	4432	1					
3	C7	Blank	3700 nt to 4800 nt	39.4	3929	1.54					
1	A8	Blank	3700 nt to 4800 nt	1.8	3897	1.42	Blank	1.77	1.75	99.07	FAIL
2	B8	Blank	3700 nt to 4800 nt	3.5	4240	2.93					
3	C8	Blank	3700 nt to 4800 nt	0	NaN	NaN					
1	A9	Blank	3700 nt to 4800 nt	15.4	4164	2.01	Blank	6.80	7.86	115.53	FAIL
2	B9	Blank	3700 nt to 4800 nt	5	3959	6.81					
3	C9	Blank	3700 nt to 4800 nt	0	NaN	NaN					
1	A10	Blank	3700 nt to 4800 nt	45.8	4044	2.18	Blank	39.00	19.13	49.05	FAIL
2	B10	Blank	3700 nt to 4800 nt	53.8	4120	2.94					
3	C10	Blank	3700 nt to 4800 nt	17.4	4081	1.24					
1	A11	GM6363-2301000423		\$47			GM6363-2301000423	\$47			PASS
2	B11	GM6363-2301000423									
3	C11	GM6363-2301000423									
1	D1	Blank	3700 nt to 4800 nt	63.3	4107	2.03	Blank	24.40	34.05	139.55	FAIL
2	D2	Blank	3700 nt to 4800 nt	9.9	3967	2.56					
3	D3	Blank	3700 nt to 4800 nt	0	NaN	NaN					
1	D4	Blank	3700 nt to 4800 nt	7.7	3968	5.43	Blank	2.57	4.45	173.21	FAIL
2	D5	Blank	3700 nt to 4800 nt	0	NaN	NaN					
3	D6	Blank	3700 nt to 4800 nt	0	NaN	NaN					
1	D7	Blank	3700 nt to 4800 nt	0	NaN	NaN	Blank	2.20	3.81	173.21	FAIL
2	D8	Blank	3700 nt to 4800 nt	0	NaN	NaN					
3	D9	Blank	3700 nt to 4800 nt	6.6	3869	4.79					

							% LATE MIGRATING SPECIES SUMMARY				COMMENTS
REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	Sample ID	Average	stdev	%CV	
1	A1	Blank	4800 nt to 13000 nt	0.4	9438	2.84	Blank	0.13	0.23	173.21	
2	B1	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C1	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A2	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
2	B2	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C2	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A3	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
2	B3	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C3	Blank	4800 nt to 13000 nt	0	9099	0.12					
1	A4	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
2	B4	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C4	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A5	Blank	4800 nt to 13000 nt	0	7561	0.15	Blank	0.00	0.00	#DIV/0!	
2	B5	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C5	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A6	Blank	4800 nt to 13000 nt	0.2	7872	3.87	Blank	0.07	0.12	173.21	
2	B6	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C6	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A7	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
2	B7	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C7	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A8	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
2	B8	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C8	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A9	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
2	B9	Blank	4800 nt to 13000 nt	0	NaN	NaN					
3	C9	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A10	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	0.00	0.00	#DIV/0!	
2	B10	Blank	4800 nt to 13000 nt	0	NaN	NaN					

3	C10	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	A11	GM6363-2301000423	4800 nt to 13000 nt	0	NaN	NaN					
2	B11	GM6363-2301000423	4800 nt to 13000 nt	0	NaN	NaN					
3	C11	GM6363-2301000423	4800 nt to 13000 nt	0	NaN	NaN					
1	D1	Blank	4800 nt to 13000 nt	0	NaN	NaN					
2	D2	Blank	4800 nt to 13000 nt	6.6	5630	14.23	Blank	2.20	3.81	173.21	
3	D3	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	D4	Blank	4800 nt to 13000 nt	5.8	5731	29.36					
2	D5	Blank	4800 nt to 13000 nt	2	10686	0.15	Blank	2.60	2.95	113.31	
3	D6	Blank	4800 nt to 13000 nt	0	NaN	NaN					
1	D7	Blank	4800 nt to 13000 nt	0	NaN	NaN					
2	D8	Blank	4800 nt to 13000 nt	0	NaN	NaN	Blank	0.67	1.15	173.21	
3	D9	Blank	4800 nt to 13000 nt	2	9175	11.41					

This tab is only to be used if a replicate needs to be excluded from the analysis.

Enter raw data directly into this table. All averages/ pass fail will be calculated automatically using the pass/fail parameters entered in the Calculations tab.

REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS	
							Sample ID	Average	stdev	%CV		
1							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

Product specific parameters for Pass/Fail		
Pass/Fail Parameters		
minimum	cut off	maximum
\$47		
result >>	\$47	

REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
							Sample ID	Average	stdev	%CV	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	
1							0	#DIV/0!	#DIV/0!	#DIV/0!	
2							0	#DIV/0!	#DIV/0!	#DIV/0!	
3							0	#DIV/0!	#DIV/0!	#DIV/0!	





VALIDATION DATA						
REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV
1	A1	sample1-rep1	3500 nt to 5389 nt	10	4079	2
2	B1	sample1-rep2	3500 nt to 5389 nt	11	4091	2.2
3	C1	sample1-rep3	3500 nt to 5389 nt	12	4089	2.4
1	A2	sample2-rep1	3500 nt to 5389 nt	20	4053	4
2	B2	sample2-rep2	3500 nt to 5389 nt	21	4061	4.2
3	C2	sample2-rep3	3500 nt to 5389 nt	22	4065	4.4
1	A3	sample3-rep1	3500 nt to 5389 nt	30	4045	6
2	B3	sample3-rep2	3500 nt to 5389 nt	31	4033	6.2
3	C3	sample3-rep3	3500 nt to 5389 nt	32	4037	6.4
1	A4	sample4-rep1	3500 nt to 5389 nt	40	4089	8
2	B4	sample4-rep2	3500 nt to 5389 nt	41	4069	8.2
3	C4	sample4-rep3	3500 nt to 5389 nt	42	4061	8.4
1	A5	sample5-rep1	3500 nt to 5389 nt	50	4061	10
2	B5	sample5-rep2	3500 nt to 5389 nt	51	4067	10.2
3	C5	sample5-rep3	3500 nt to 5389 nt	52	4070	10.4
1	A6	sample6-rep1	3500 nt to 5389 nt	60	4009	12
2	B6	sample6-rep2	3500 nt to 5389 nt	61	3998	12.2
3	C6	sample6-rep3	3500 nt to 5389 nt	62	4097	12.4
1	A7	sample7-rep1	3500 nt to 5389 nt	70	4071	14
2	B7	sample7-rep2	3500 nt to 5389 nt	71	4049	14.2
3	C7	sample7-rep3	3500 nt to 5389 nt	72	4060	14.4

REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV
1	A1	sample1-rep1	5389 nt to 13000 nt	1	6774	0.2
2	B1	sample1-rep2	5389 nt to 13000 nt	2	5534	0.42
3	C1	sample1-rep3	5389 nt to 13000 nt	3	5684	0.64
1	A2	sample2-rep1	5389 nt to 13000 nt	2	6916	0.4
2	B2	sample2-rep2	5389 nt to 13000 nt	3	4079	0.62
3	C2	sample2-rep3	5389 nt to 13000 nt	4	5530	0.84
1	A3	sample3-rep1	5389 nt to 13000 nt	3	6870	0.6
2	B3	sample3-rep2	5389 nt to 13000 nt	4	6807	0.82
3	C3	sample3-rep3	5389 nt to 13000 nt	5	6551	1.04
1	A4	sample4-rep1	5389 nt to 13000 nt	4	7320	0.8
2	B4	sample4-rep2	5389 nt to 13000 nt	5	7000	1.02
3	C4	sample4-rep3	5389 nt to 13000 nt	6	6970	1.24
1	A5	sample5-rep1	5389 nt to 13000 nt	5	7135	1
2	B5	sample5-rep2	5389 nt to 13000 nt	6	7094	1.22
3	C5	sample5-rep3	5389 nt to 13000 nt	7	6740	1.44
1	A6	sample6-rep1	5389 nt to 13000 nt	6	4079	1.2
2	B6	sample6-rep2	5389 nt to 13000 nt	7	5436	1.42
3	C6	sample6-rep3	5389 nt to 13000 nt	8	8653	1.64
1	A7	sample7-rep1	5389 nt to 13000 nt	7	7717	1.4
2	B7	sample7-rep2	5389 nt to 13000 nt	8	7570	1.62
3	C7	sample7-rep3	5389 nt to 13000 nt	9	8404	1.84

RESULTS FOR VALIDATION DATA											
REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	% INTEGRITY SUMMARY				COMMENTS
							Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	3500 nt to 5389 nt	10	4079	2	sample1-rep1	11.00	1.00	9.09	FAIL
2	B1	sample1-rep2	3500 nt to 5389 nt	11	4091	2.2					
3	C1	sample1-rep3	3500 nt to 5389 nt	12	4089	2.4					
1	A2	sample2-rep1	3500 nt to 5389 nt	20	4053	4	sample2-rep1	21.00	1.00	4.76	FAIL
2	B2	sample2-rep2	3500 nt to 5389 nt	21	4061	4.2					
3	C2	sample2-rep3	3500 nt to 5389 nt	22	4065	4.4					
1	A3	sample3-rep1	3500 nt to 5389 nt	30	4045	6	sample3-rep1	31.00	1.00	3.23	FAIL
2	B3	sample3-rep2	3500 nt to 5389 nt	31	4033	6.2					
3	C3	sample3-rep3	3500 nt to 5389 nt	32	4037	6.4					
1	A4	sample4-rep1	3500 nt to 5389 nt	40	4089	8	sample4-rep1	41.00	1.00	2.44	FAIL
2	B4	sample4-rep2	3500 nt to 5389 nt	41	4069	8.2					
3	C4	sample4-rep3	3500 nt to 5389 nt	42	4061	8.4					
1	A5	sample5-rep1	3500 nt to 5389 nt	50	4061	10	sample5-rep1	51.00	1.00	1.96	FAIL
2	B5	sample5-rep2	3500 nt to 5389 nt	51	4067	10.2					
3	C5	sample5-rep3	3500 nt to 5389 nt	52	4070	10.4					
1	A6	sample6-rep1	3500 nt to 5389 nt	60	4009	12	sample6-rep1	61.00	1.00	1.64	PASS
2	B6	sample6-rep2	3500 nt to 5389 nt	61	3998	12.2					
3	C6	sample6-rep3	3500 nt to 5389 nt	62	4097	12.4					
1	A7	sample7-rep1	3500 nt to 5389 nt	70	4071	14	sample7-rep1	71.00	1.00	1.41	PASS
2	B7	sample7-rep2	3500 nt to 5389 nt	71	4049	14.2					
3	C7	sample7-rep3	3500 nt to 5389 nt	72	4060	14.4					

REPLICATE	Well	Sample ID	Range	% Total	Avg. Size	%CV	% LATE MIGRATING SPECIES SUMMARY				COMMENTS
							Sample ID	Average	stdev	%CV	
1	A1	sample1-rep1	5389 nt to 13000 nt	1	6774	0.2	sample1-rep1	2.00	1.00	50.00	
2	B1	sample1-rep2	5389 nt to 13000 nt	2	5534	0.42					
3	C1	sample1-rep3	5389 nt to 13000 nt	3	5684	0.64					
1	A2	sample2-rep1	5389 nt to 13000 nt	2	6916	0.4	sample2-rep1	3.00	1.00	33.33	
2	B2	sample2-rep2	5389 nt to 13000 nt	3	4079	0.62					
3	C2	sample2-rep3	5389 nt to 13000 nt	4	5530	0.84					
1	A3	sample3-rep1	5389 nt to 13000 nt	3	6870	0.6	sample3-rep1	4.00	1.00	25.00	
2	B3	sample3-rep2	5389 nt to 13000 nt	4	6807	0.82					
3	C3	sample3-rep3	5389 nt to 13000 nt	5	6551	1.04					
1	A4	sample4-rep1	5389 nt to 13000 nt	4	7320	0.8	sample4-rep1	5.00	1.00	20.00	
2	B4	sample4-rep2	5389 nt to 13000 nt	5	7000	1.02					
3	C4	sample4-rep3	5389 nt to 13000 nt	6	6970	1.24					
1	A5	sample5-rep1	5389 nt to 13000 nt	5	7135	1	sample5-rep1	6.00	1.00	16.67	
2	B5	sample5-rep2	5389 nt to 13000 nt	6	7094	1.22					
3	C5	sample5-rep3	5389 nt to 13000 nt	7	6740	1.44					
1	A6	sample6-rep1	5389 nt to 13000 nt	6	4079	1.2	sample6-rep1	7.00	1.00	14.29	
2	B6	sample6-rep2	5389 nt to 13000 nt	7	5436	1.42					
3	C6	sample6-rep3	5389 nt to 13000 nt	8	8653	1.64					
1	A7	sample7-rep1	5389 nt to 13000 nt	7	7717	1.4	sample7-rep1	8.00	1.00	12.50	
2	B7	sample7-rep2	5389 nt to 13000 nt	8	7570	1.62					
3	C7	sample7-rep3	5389 nt to 13000 nt	9	8404	1.84					

## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2023 02 16\12-01-33\2023 02 16 12H 01M.raw

**Created:** Thursday, February 16, 2023 12:27:34 PM

**Number of capillaries:** 8

**Array serial number:** 031221-22SFS

**Effect length:** 33 cm

**Array usage count:** 13

**Instrument type:** 5300 Fragment Analyzer

**Instrument controller software version:** 3.1.0.12

**Device serial number:** MY2105AB19

## Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds

**Gel prime:** No

**Full conditioning:** Yes

**Gel prime to buffer:** Yes

**Gel selection:** Gel 2

**Perform prerun:** 8.0 kV, 30 sec.

**Rinse:** No

**Marker 1:** No

**Rinse:** Tray: 3, Row: A, Dip count: 2

**Sample injection:** 5.0 kV, 15 sec.

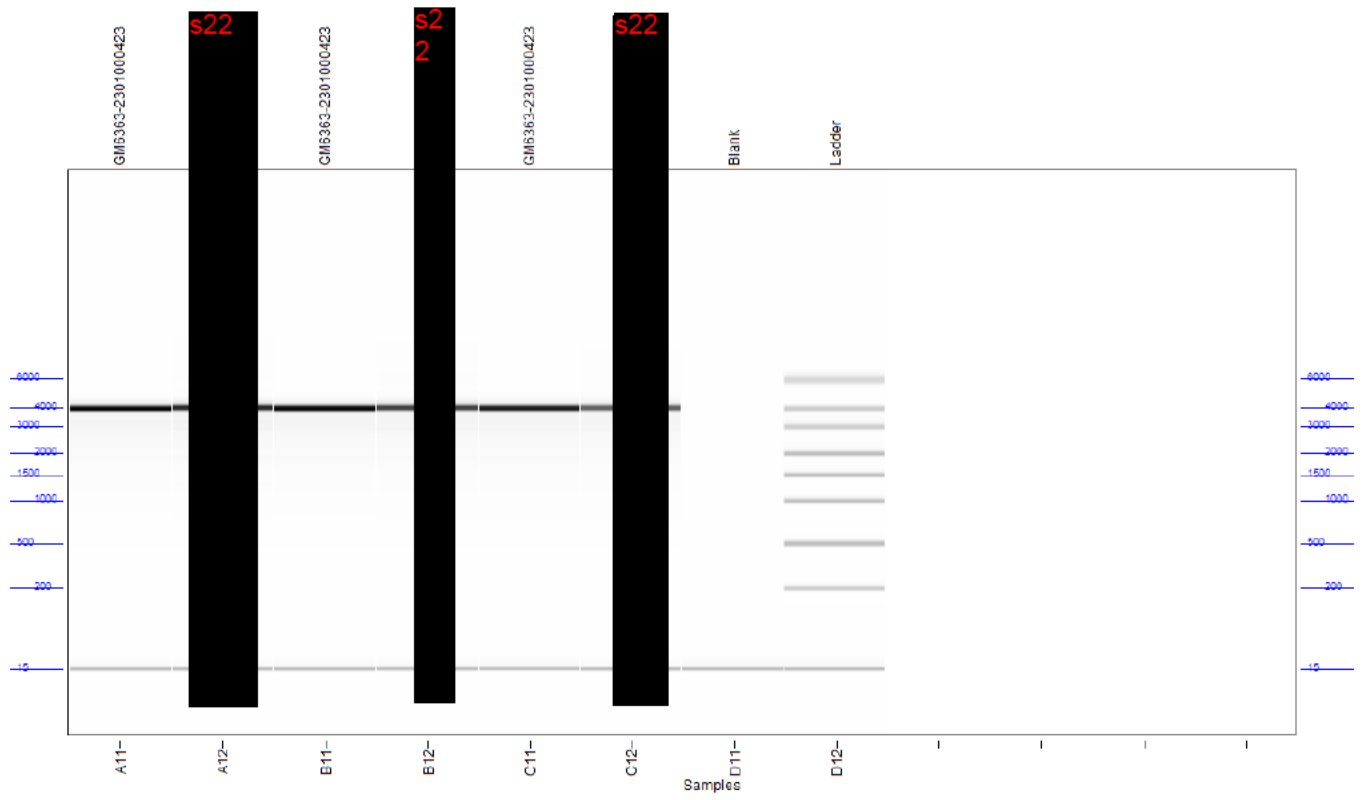
**Separation:** 8.0 kV, 60.0 min.

**Tray name:** Tray-1

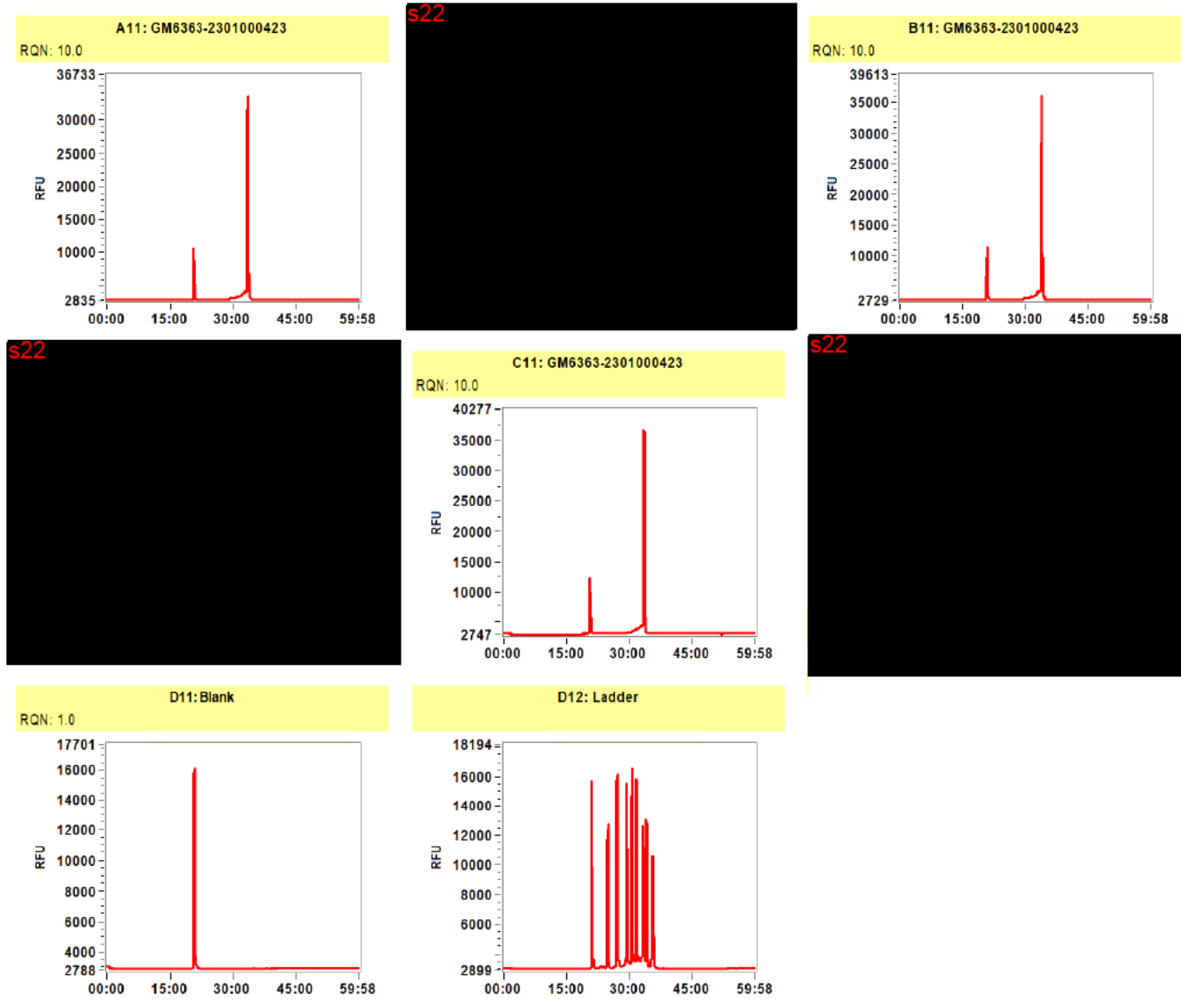
**Analysis mode:** RNA (Eukaryotic)

## Notes

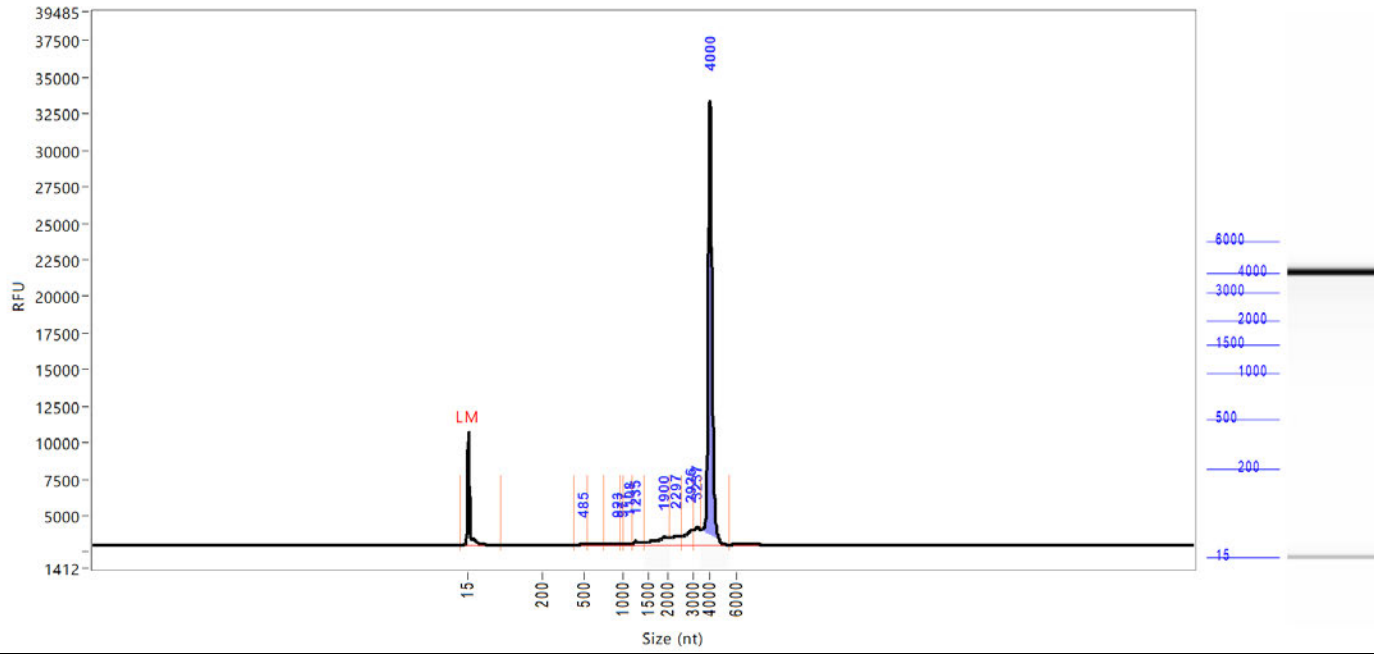
### Gel Image



Filename and data path: C:\Agilent Technologies\Data\2023 02 16\12-01-33\2023 02 16 12H 01M.raw



S22



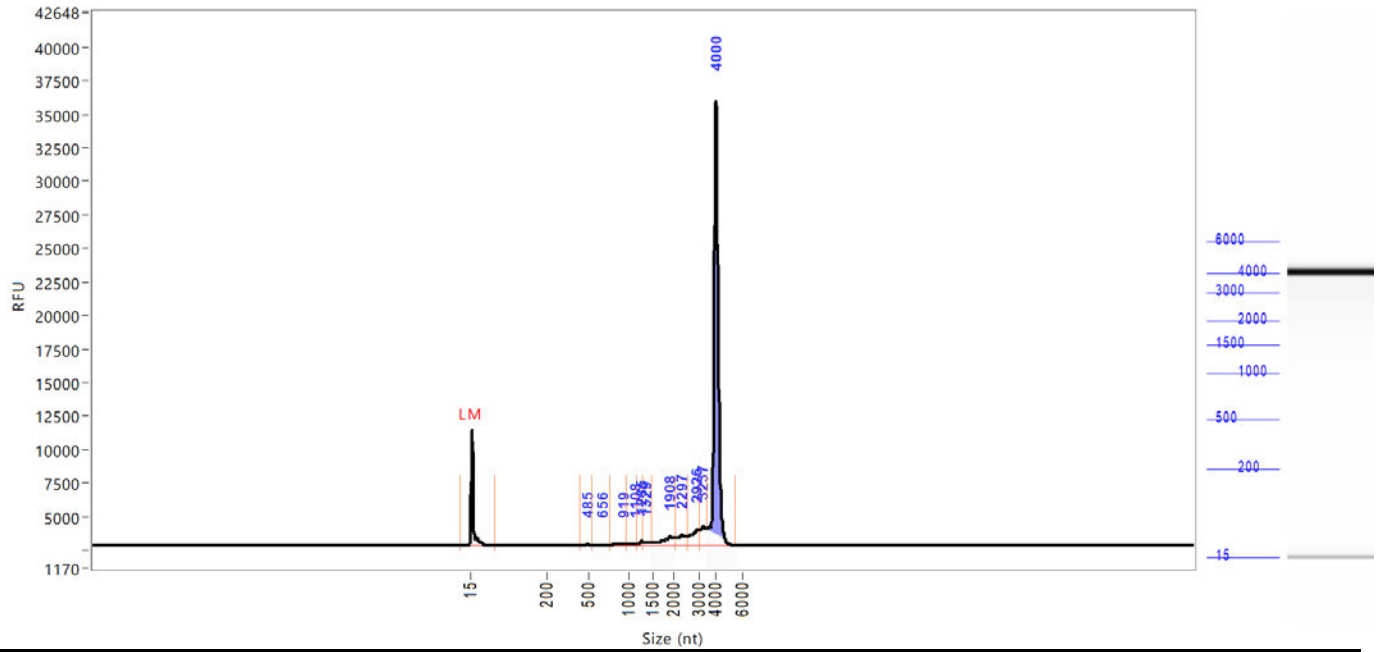
S47

s22



GM6363-2301000423

s22



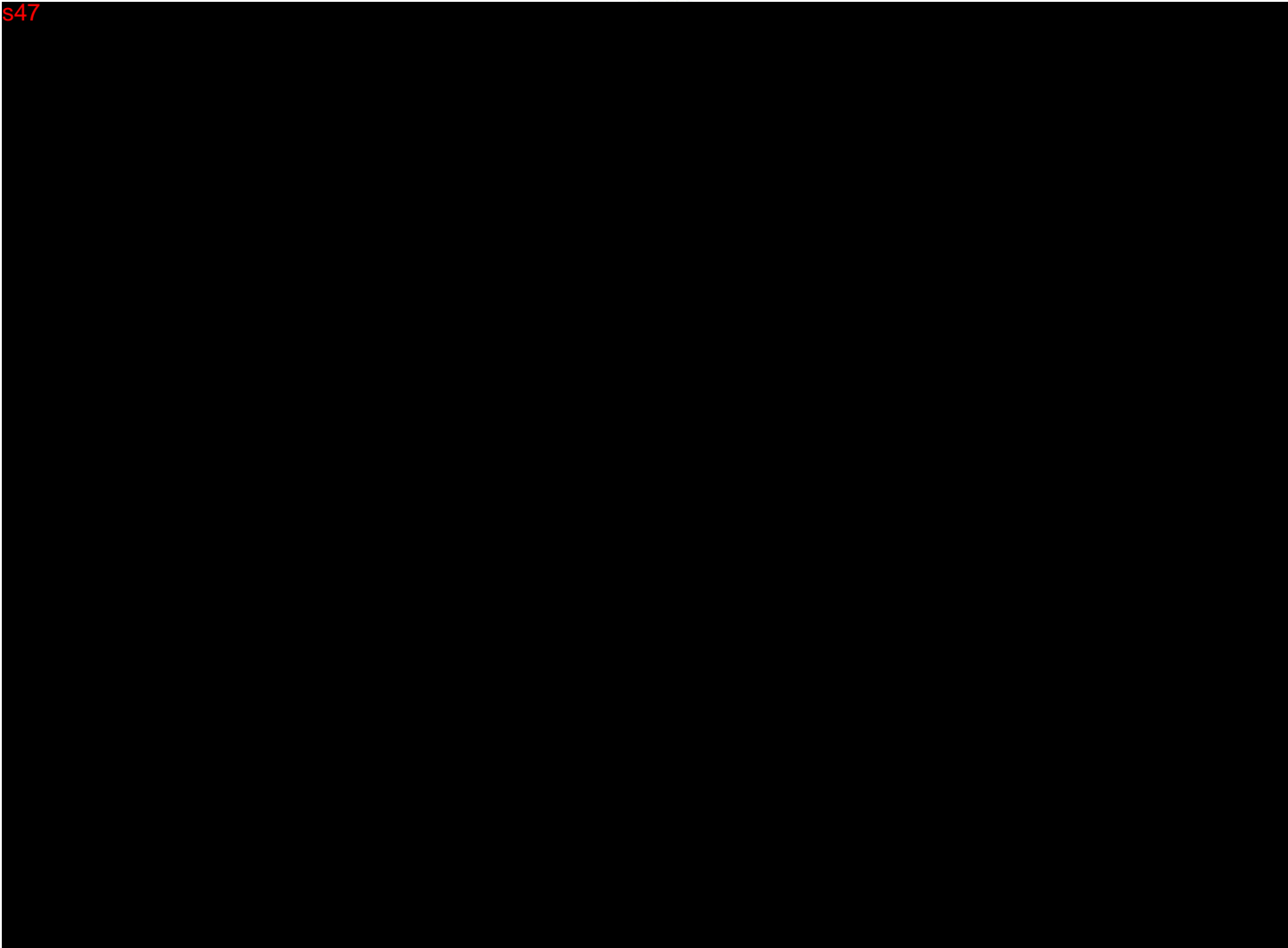
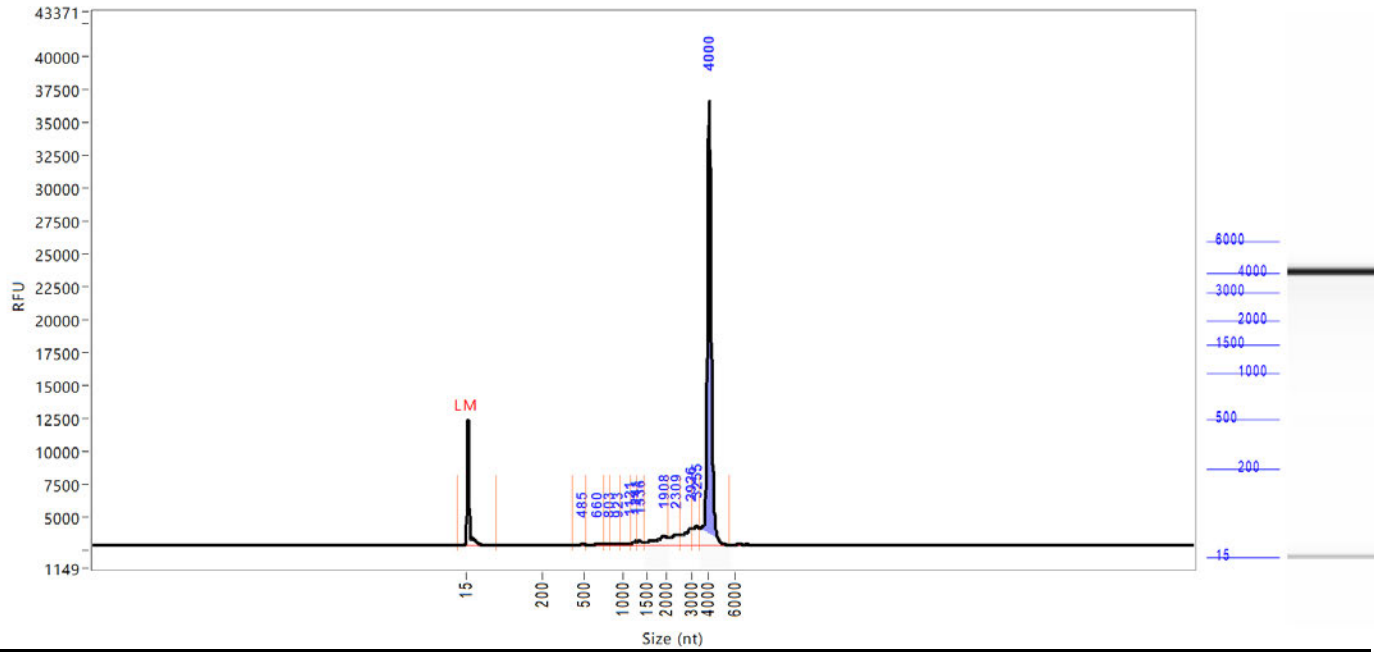
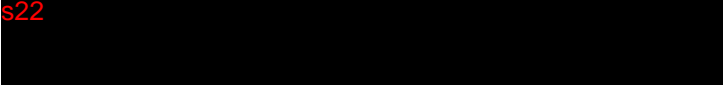
s47



s22



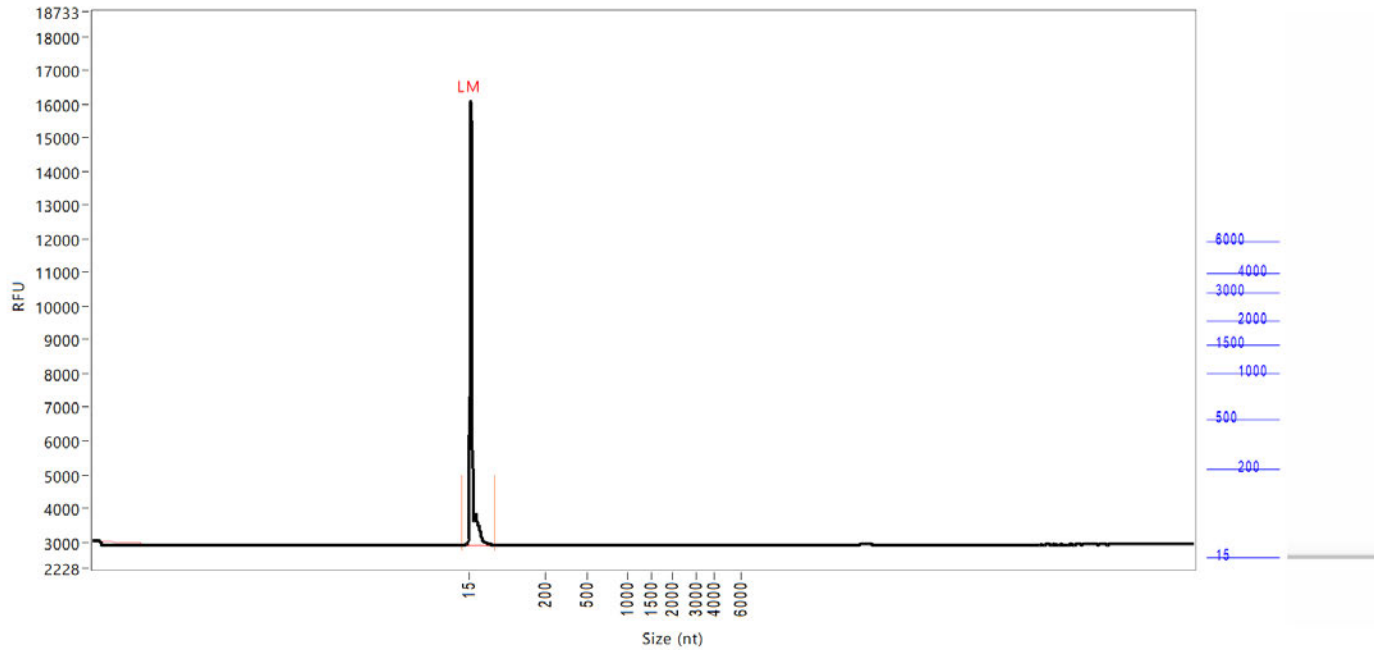
Sample: GM6363-2301000423



s22



**Sample:** Blank  
**Well location:** D11  
**Created:** Thursday, February 16, 2023 12:27:34 PM

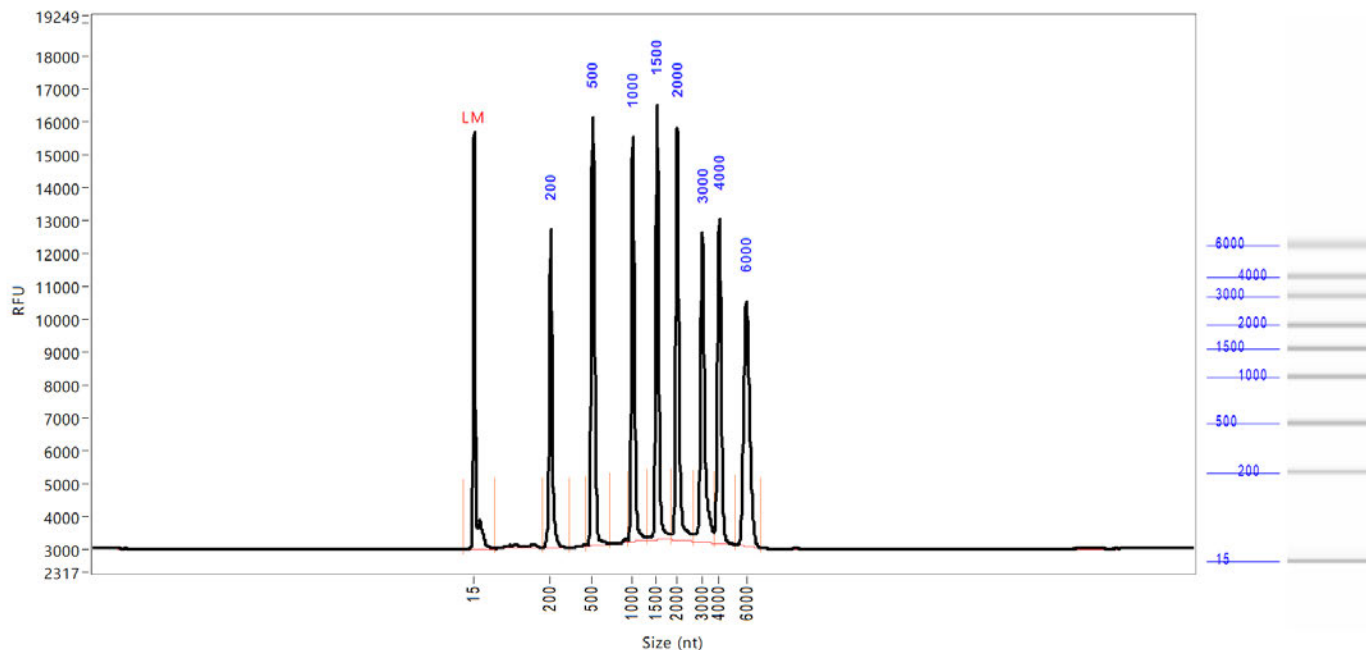


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	1.1174	0	76	13193
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0051	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	3700 nt to 4800 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV
	4800 nt to 13000 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Thursday, February 16, 2023 12:27:34 PM



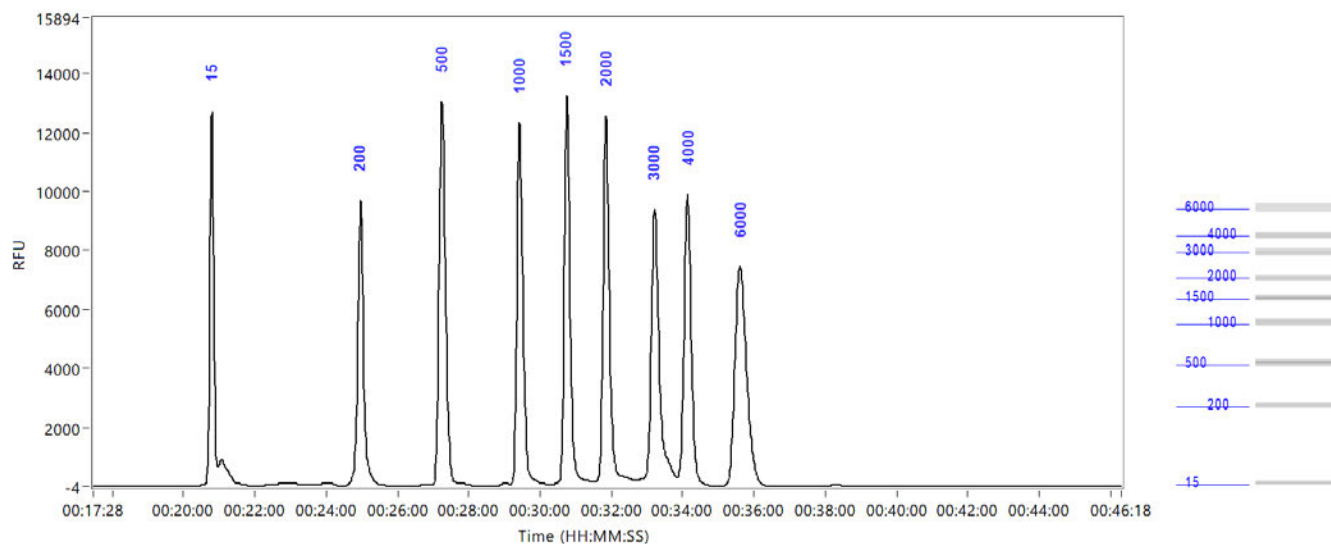
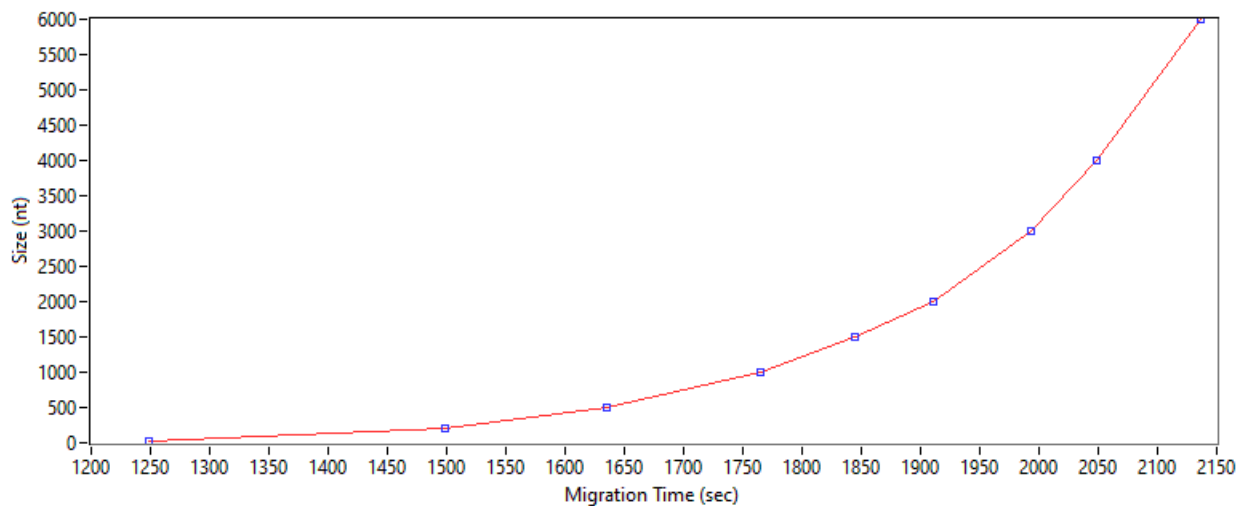
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	1.1174	0	67	12704
2	200	10.7471	179	331	9688
3	500	14.9990	458	721	13030
4	1000	12.3653	942	1311	12312
5	1500	12.2169	1311	1862	13243
6	2000	12.3699	1862	2642	12559
7	3000	11.4036	2642	3673	9416
8	4000	10.0162	3673	5196	9887
9	6000	11.7306	5196	7114	7471

TIC: 95.8486 ng/uL  
 TIM: 365.3386 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6    Sample min peak height: 200    Sample baseline V to V?: Y    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Thursday, February 16, 2023 12:27:34 PM  
**Fit type:** Point to point

Calibration curve





<b>Type:</b> Biotherapeutics\BPC\Forms	<b>Number:</b> Bio-BPC-Form-10 / <b>Version:</b> 2
<b>Owner:</b> s22	<b>Approver:</b> s22
<b>Active:</b> 14/11/2022	<b>Review:</b> 20/07/2022
<b>Title:</b> HPLC – General Worksheet	

## HPLC General Worksheet

TEST DETAILS			
TEST NAME	Analysis of mRNA Purity in Comirnaty (Pfizer) bivalent vaccine by Size-based RPIP		
METHOD REFERENCE	Bio-BPC-Method-33 (s47) – as a part of method development study (D22-6079971)		
METHOD MODIFICATIONS (if any)	Use of Pfizer DP RM		
MODIFICATIONS APPROVED BY:	s22		
DATA LOCATION IN TRIM	E22-628460, D23-5145305		
NAME OF ANALYST	s22	TEST DATE	21/02/2023

BUFFERS AND SOLUTIONS	
SOLUTIONS	BATCH No:
Mobile phase A	CP20Feb23-1
Mobile phase B	CP20Feb23-2
SAMPLE DILUENT	N/A
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Click or tap here to enter text.	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.

PIPETTES USED AND EXPIRY DATES	
LIMS# 33440	21/05/2023
LIMS# 33438	21/05/2023
LIMS# 33367	3/04/2023
	Click or tap to enter a date.

REFERENCE MATERIALS	
NAME AND CODE	BATCH NO:
<b>Pfizer Reference Material</b>	<b>EL8983</b>
Click or tap to enter a date.	Click or tap to enter a date.
Click or tap to enter a date.	Click or tap to enter a date.
Click or tap to enter a date.	Click or tap to enter a date.
Click or tap to enter a date.	Click or tap to enter a date.
Click or tap to enter a date.	Click or tap to enter a date.

Reference Material Concentration = 0.47 ug/mL

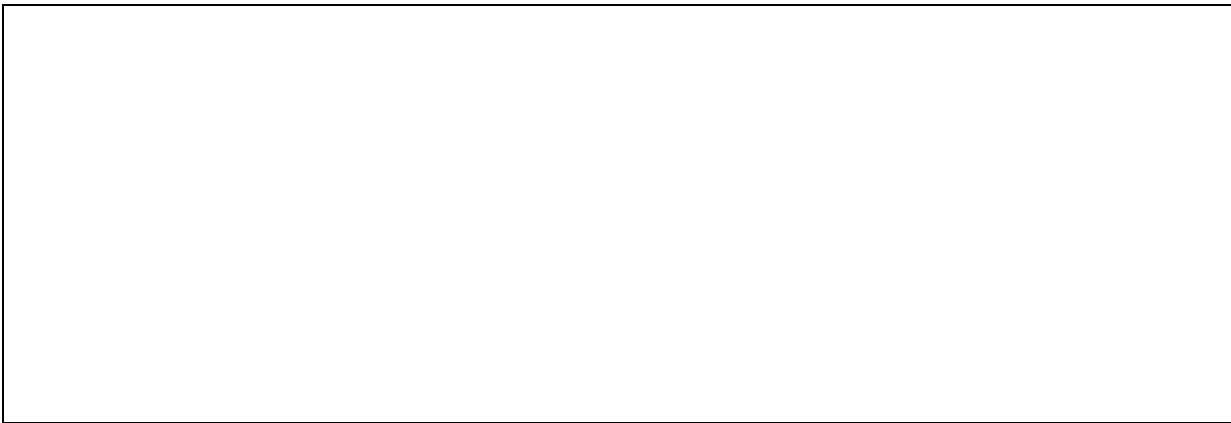
Required Concentration = 0.2 mg/mL

Diluted 85 uL of the reference material in 115 uL of RNase free water to make 0.2mg/ml.

Sensitivity Solution= 0.002mg/mL

Diluted 10uL of the 0.2mg/mL solution in 990uL of RNase free water to make 0.002mg/mL.





<b>SYSTEM SUITABILITY CRITERIA AND RESULTS</b>			
<b>PARAMETERS</b>	<b>LIMITS</b>	<b>RESULTS</b>	<b>COMMENTS</b>
No peak in the water blank injection prior to the Sensitivity Solution	No peak	No peak	PASS
All other carryover water blank injections in the sequence interfere with the peaks of interest as compared to the total peak area of the mRNA working reference standard	Interference $\leq 1\%$	Interfering peaks $< 1\%$	PASS
Signal to Noise ratio of Sensitivity Solution	$\geq 10$	10.68	PASS
% Recovery of the main peak area in each bracketing standard compared to the peak area of the mRNA working reference standard	90-110%	103.8	PASS
% Agreement of the main peak RT in each bracketing standard compared to the main peak RT of the mRNA working reference standard	90-110%	103.05	PASS
Absolute Difference of the Main Peak % Area for duplicate sample preparations - 2301000423	$\leq 5\%$	-1.766	PASS
Absolute Difference of the Main Peak % Area for duplicate sample preparations - 2301000435	$\leq 5\%$	0.832	PASS
Absolute Difference of the Main Peak % Area for duplicate sample preparations - 2301000437	$\leq 5\%$	2.100	PASS
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SAMPLE DETAILS					
SAMPLE NAME	COMIRNATY ORIGINAL/OMICRON BA.4-5 (tozinameran/famtozinameran) COVID-19 VACCINE 15/15 micrograms/0.3 mL suspension for injection vial				
LIMS No:	2301000423	BATCH No:	GM6363		
SAMPLE DILUTIONS, CALCULATIONS					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
0.1 mg/mL	100 uL	-	0.1 mg/mL	-	10 uL
No dilution, 100 uL of neat sample taken into UPLC total recovery vial for injection.					

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	COMMENTS
IG:RNA Fragments	N/A	s47	
IG:Lipid adducts	N/A		
Main Peak	s47		
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<b>SAMPLE DETAILS</b>					
SAMPLE NAME	COMIRNATY ORIGINAL/OMICRON BA.4-5 (tozinameran/famtozinameran) COVID-19 VACCINE 15/15 micrograms/0.3 mL suspension for injection vial				
LIMS No:	2301000435	BATCH No:	s22		
<b>SAMPLE DILUTIONS, CALCULATIONS</b>					
<b>Initial Conc.</b>	<b>Vol. sample</b>	<b>Vol. diluent</b>	<b>Final Conc.</b>	<b>DF</b>	<b>Inj. Vol.</b>
0.1 mg/ml	100 uL	-	0.1 mg/mL	-	10 uL
No dilution, 100 uL of neat sample taken into UPLC total recovery vial for injection.					

<b>TEST RESULTS</b>			
PARAMETERS	LIMITS	RESULTS	COMMENTS
IG:RNA Fragments	s22		
IG:Lipid adducts			
Main Peak	s47	s22	
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Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.		
<b>SAMPLE DETAILS</b>					
SAMPLE NAME	COMIRNATY ORIGINAL/OMICRON BA.4-5 (tozinameran/famtozinameran) COVID-19 VACCINE 15/15 micrograms/0.3 mL suspension for injection vial				
LIMS No:	2301000437	BATCH No:	s22		
<b>SAMPLE DILUTIONS, CALCULATIONS</b>					
<b>Initial Conc.</b>	<b>Vol. sample</b>	<b>Vol. diluent</b>	<b>Final Conc.</b>	<b>DF</b>	<b>Inj. Vol.</b>
0.1 mg/mL	100 uL	-	0.1 mg/mL	-	10 uL
No dilution, 100 uL of neat sample taken into UPLC total recovery vial for injection.					

<b>TEST RESULTS</b>			
PARAMETERS	LIMITS	RESULTS	COMMENTS
IG:RNA Fragments	s22		
IG:Lipid adducts			
Main Peak	s47 s22		
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**SAMPLE DETAILS**

SAMPLE NAME	Click or tap here to enter text.		
LIMS No:	Click or tap here to enter text.	BATCH No:	Click or tap here to enter text.

**SAMPLE DILUTIONS, CALCULATIONS**

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.

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**TEST RESULTS**

PARAMETERS	LIMITS	RESULTS	COMMENTS
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**SAMPLE DETAILS**

SAMPLE NAME	Click or tap here to enter text.		
LIMS No:	Click or tap here to enter text.	BATCH No:	Click or tap here to enter text.

**SAMPLE DILUTIONS, CALCULATIONS**

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
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**TEST RESULTS**

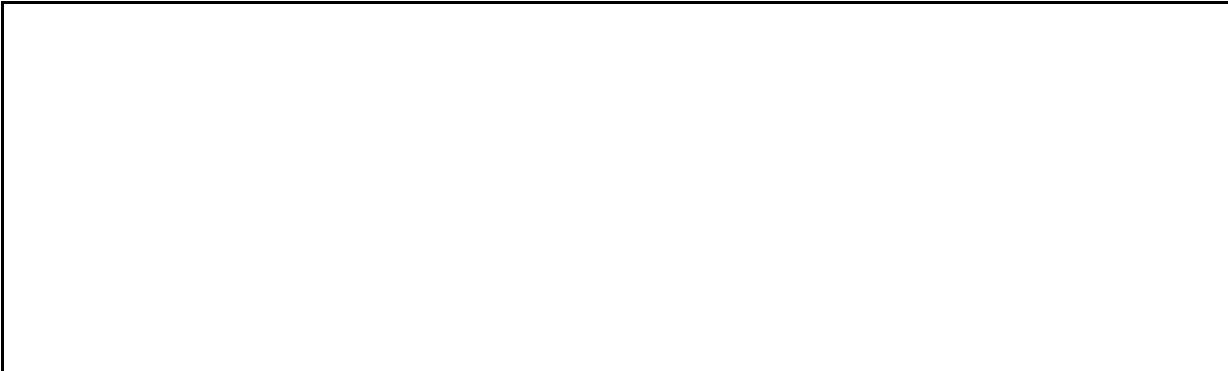
PARAMETERS	LIMITS	RESULTS	COMMENTS
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**NOTES**

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<b>Type:</b> Biotherapeutics\BEE\Forms	<b>Number:</b> Bio-BEE-Form-39 / <b>Version:</b> 3
<b>Owner:</b> s22	<b>Approver:</b> s22
<b>Active:</b> 10/05/2022	<b>Review:</b> 10/11/2023
<b>Title:</b> Endotoxin Routine Assay Worksheet	

## Endotoxin Routine Assay Worksheet

Assay ID: 15Feb2023 Operator: s22

### Limulus Amoebocyte Lysate (LAL)

Lysate batch and expiry recorded on software for each assay

Ensure sensitivity of LAL batch has been confirmed. 'Lysate preparation details' shown below

### Recombinant Factor C (rFC)

rFC Enzyme, Fluorogenic Substrate & rFC Assay Buffer batches and expiry dates recorded on software for each assay

Ensure sensitivity of the rFC batch has been confirmed. 'rFC Reagent preparation details' shown below

### Control Standard Endotoxin (CSE) – refer to Bio-BEE-Method 5 and Bio-BEE-Form 37

CSE batch and expiry recorded on software for each assay

Reconstitution details for either KLAL or rFC – see Trim File D23-5062784

CSE Lot Number: 0000981165 Conc 50 EU/mL

### LAL Reagent Water (LRW) Lot Number: 0000981166 Expiry: 16Sep2023

How many samples were linked to this assay? 5

**This form is used for recording the assay details and results and only gives the method in point form. See Bio-BEE-SOP 28 and appropriate method for the detailed procedure.**

To avoid endotoxin contamination, use careful technique and **pyrogen free** equipment.

### Preparation of Assay

- Fill out the appropriate forms from the Quality Management System (QMS)
- Retrieve the required kit reagents from cold storage to equilibrate to room temperature
- Turn on plate reader and computer and follow the steps as detailed in Bio-BEE-Method 4

### Preparation of CSE (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare CSE as detailed in Bio-BEE-Method 5. CSE dilutions can be dispensed to the plate as they are prepared to save mixing time

- CSE is set up as in the table below - record %CV results from the final report

#### For a KLAL (KQCL) assay

Concentration	Plate wells ID	% CV
50 EU/ml	F1 – F2	2.13
5 EU/ml	E1 – E2	1.82
0.5 EU/ml	D1 – D2	1.80
0.05 EU/ml	C1 – C2	0.91
0.005 EU/ml	B1 – B2	0.96
Blank	A1 – A2	

#### For an rFC assay

Concentration	Plate wells ID	% CV
5 EU/ml	E1 – E2	N/A
0.5 EU/ml	D1 – D2	N/A
0.05 EU/ml	C1 – C2	N/A
0.005 EU/ml	B1 – B2	N/A
Blank	A1 – A2	

- Dispense 100 µl of the appropriate dilution of CSE into the appropriate wells of the plate.
- Continue with procedure as per the appropriate method

#### Preparation of Samples (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare sample dilutions as in Bio-BEE-Form 42
- Pipette 10 µl of the 5 EU/ml standard to the appropriate PPC wells as per the plate layout
- Dispense 100 µl of the final sample dilutions into the 4 appropriate wells as per the plate layout

#### Starting the Assay

- The plate is then ready for the reaction. Prepare the software as set out in the Bio-BEE-Method 4
- “Run” the Template prepared earlier. Follow the prompts to the Pre-warming step

#### If performing a KLAL assay

- Prepare the required lysate vial/s (Bop-BEE-Method 6) and pour into the reagent reservoir

Lysate Preparations Details -

Lysate Lot Number	XL021QDD4P	Expiry 17Aug2023
Date sensitivity confirmed	09May2022	TRIM#: E22-564008
Reconstitute lysate with	2.6mL (3 x 867µL)	mL of LAL Reagent Water (LRW)
Date reconstituted	15Feb2022	1 vial
Operator(s)	s22	N/A
Use by date	01Mar2022	(Lonza KQCL- 14 days from reconstitution, at below -10°C)

**OR**

*If performing an rFC assay*

- Make up the required volume of rFC reagent directly into the reservoir (Bio-BEE-Method 6)

rFC Reagent Preparations Details – (Note: once prepared, working reagent cannot be stored)

	Lot	Expiry	Volume
Date sensitivity confirmed	N/A	N/A	
Fluorogenic Substrate	N/A	N/A	N/A µL
rFC Assay Buffer	N/A	N/A	N/A µL
rFC Enzyme Solution	N/A	N/A	N/A µL

- Open cover – if using the Spectramax use the software to open and close the drawer
- Add 100 µl of either lysate (KLAL) **OR** working reagent (rFC) to each of the assay wells, carefully, and as quickly as possible
- Close the drawer on the plate reader and click OK to start the run. **Do not open drawer**

**Acceptance Criteria – for KLAL**

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>-0.998</u>
Slope between -0.400 and -0.100	<u>-0.219</u>
Y intercept between 2.500 and 3.500	<u>3.073</u>
Mean reaction times of blank $\geq$ mean reaction times of lowest standard	<u>Yes</u>
Coefficient of variation (CV) values for all standards are $< 10\%$	<u>Yes</u>
Were all acceptance criteria for the standard curve met?	<u>Yes</u>

## Acceptance Criteria – for rFC

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>N/A</u>
Slope between 0.760 and 1.110	<u>N/A</u>
Y intercept between 2.500 and 5.000	<u>N/A</u>
Mean RFU of blank $\leq$ mean RFU of lowest standard	<u>N/A</u>
Coefficient of variation (CV) values for all standards are $< 25\%$	<u>N/A</u>
Were all acceptance criteria for the standard curve met?	<u>N/A</u>

## Conclusions

Follow procedures for 'Recording Results' detailed in Bio-BEE-Method 7

## Notes

Checked **s22** 17Feb2023



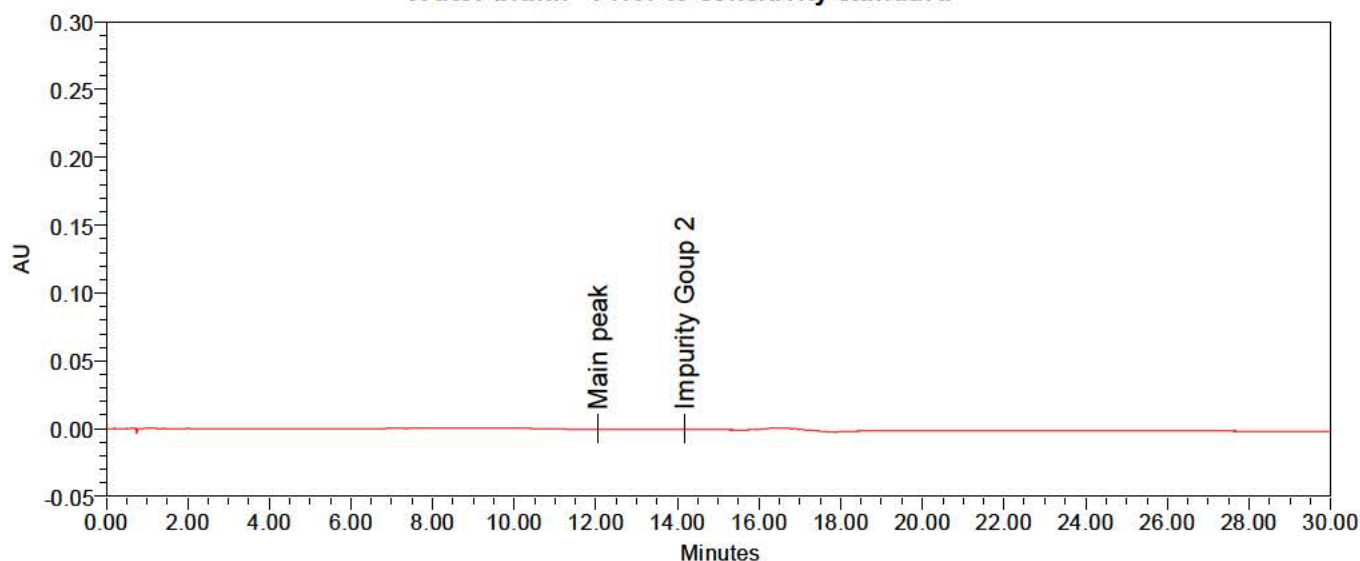
Australian Government  
Department of Health and Ageing  
Therapeutic Goods Administration

s22 [Redacted], Water Blank, Reference  
Standard, s22 [Redacted]  
2111004293-P1. water. 2111004293-P2. s22 [Redacted]

Sample Set Name: 30Nov21 3Moderna 1 Pfizer  
Sample Set Acquired By: s22 [Redacted]

## System Suitability

Water blank - Prior to sensitivity standard

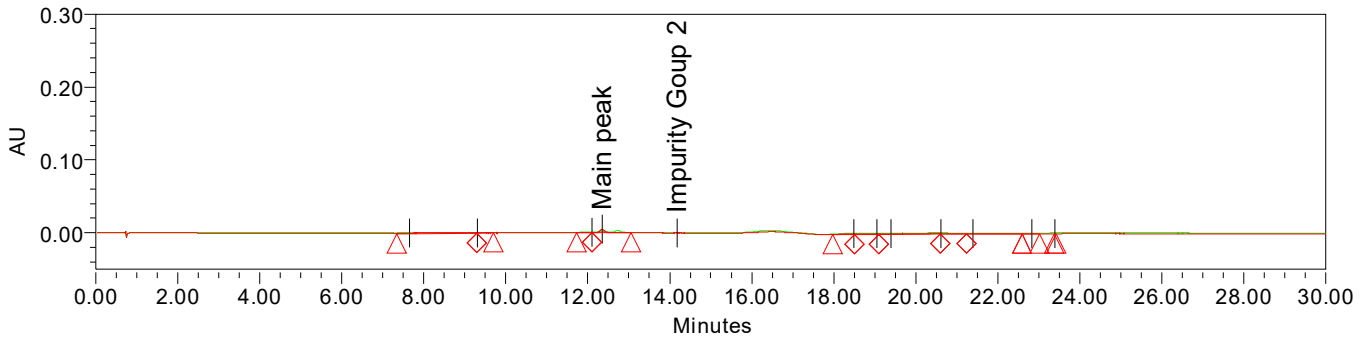


— Label B2; SampleName: Water Blank; Vial: 1:A,1; Injection: 1

Component Results

	Name	RT	Area	Height	Amount	Units	Total Area	TA_Ratio
1	Impurity group 3						0	
2	Impurity group 1						0	
3	Main peak	12.054					0	
4	Impurity Goup 2	14.173					0	

Water blanks - carryover assessment



— Label B3; SampleName: Water Blank; Injection: 1; Channel PDA Ch1 260nm@4.8nm  
 — Label B4; SampleName: Water Blank; Injection: 1; Channel PDA Ch1 260nm@4.8nm

TA\_Ratio Summarized by Name  
Label: B3

	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity group 3
	B3	Water Blank	1	254991	3.40	3.40	3.40
				254991			

TA\_Ratio Summarized by Name  
Label: B4

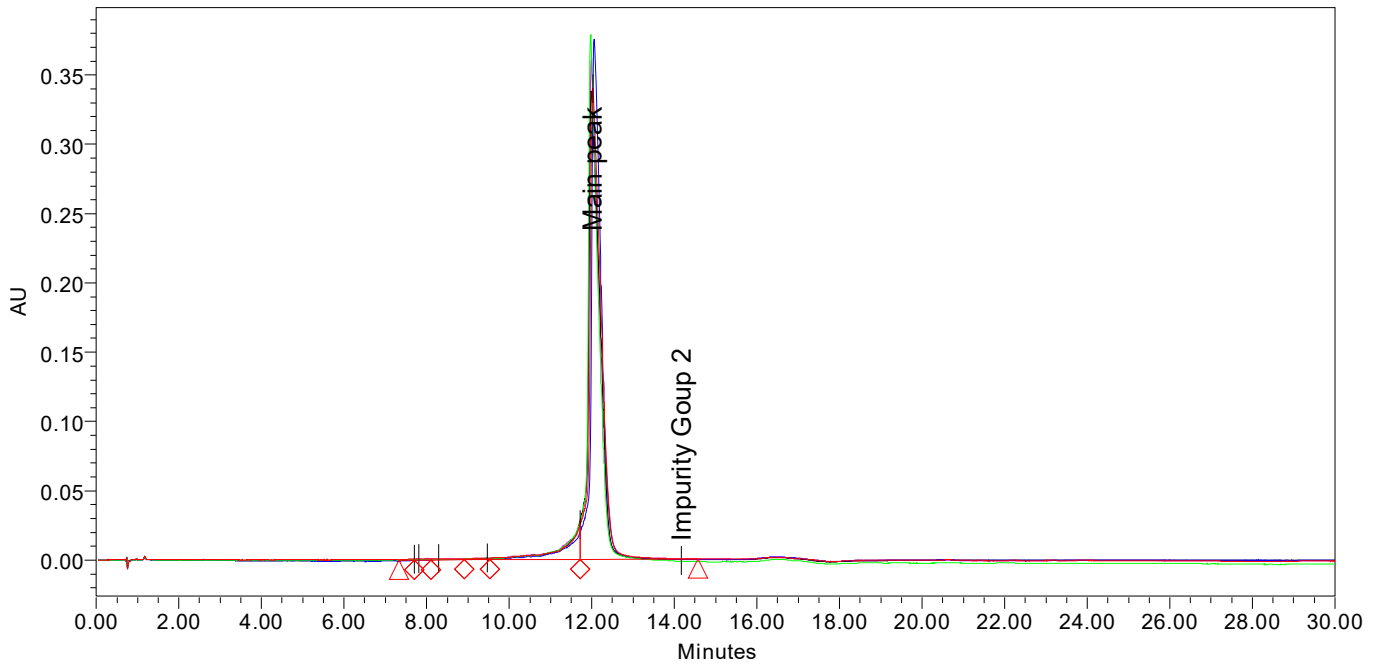
	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity group 3
	B4	Water Blank	1	285606	3.81	3.81	3.81
				285606			



s22



First five reference standard injections



- SampleName: Reference Standard; Vial: 1:A,3; Injection: 1; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 2; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 3; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 4; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 5; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm

Component Summary Table  
Name: Main peak

	Sample Name	Vial	Inj	Channel	RT	Area	Total Area
1	Reference Standard	1:A,3	1	PDA Ch1 260nm@4.8nm	12.022	6423947	7198450
2	Reference Standard	1:A,3	2	PDA Ch1 260nm@4.8nm	11.973	6638629	7817775
3	Reference Standard	1:A,3	3	PDA Ch1 260nm@4.8nm	12.054	6491888	7618336
4	Reference Standard	1:A,3	4	PDA Ch1 260nm@4.8nm	11.988	6510259	7525577
5	Reference Standard	1:A,3	5	PDA Ch1 260nm@4.8nm	11.991	6338383	7319557
Mean					12.006	6480621	7495939
Std. Dev.					0.032	111148	244462.4
% RSD					0.3	1.7	3.3

S22



## Sample Acceptance Criteria

TA\_Ratio Summarized by Name  
Label: U1

	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity Goup 2	Impurity group 3
1	U1	2111004293-P1	1	6169911	82.31	82.31	82.31	82.31
2	U1	2111004293-P2	1	5567562	74.27	74.27	74.27	74.27
Mean				5868736				
Std. Dev.				425926				
% RSD				7.3				

TA\_Ratio Summarized by Name  
Label: U2

	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity Goup 2	Impurity group 3
1	U2	s47						
2	U2							
Mean								
Std. Dev.								
% RSD								

TA\_Ratio Summarized by Name  
Label: U3

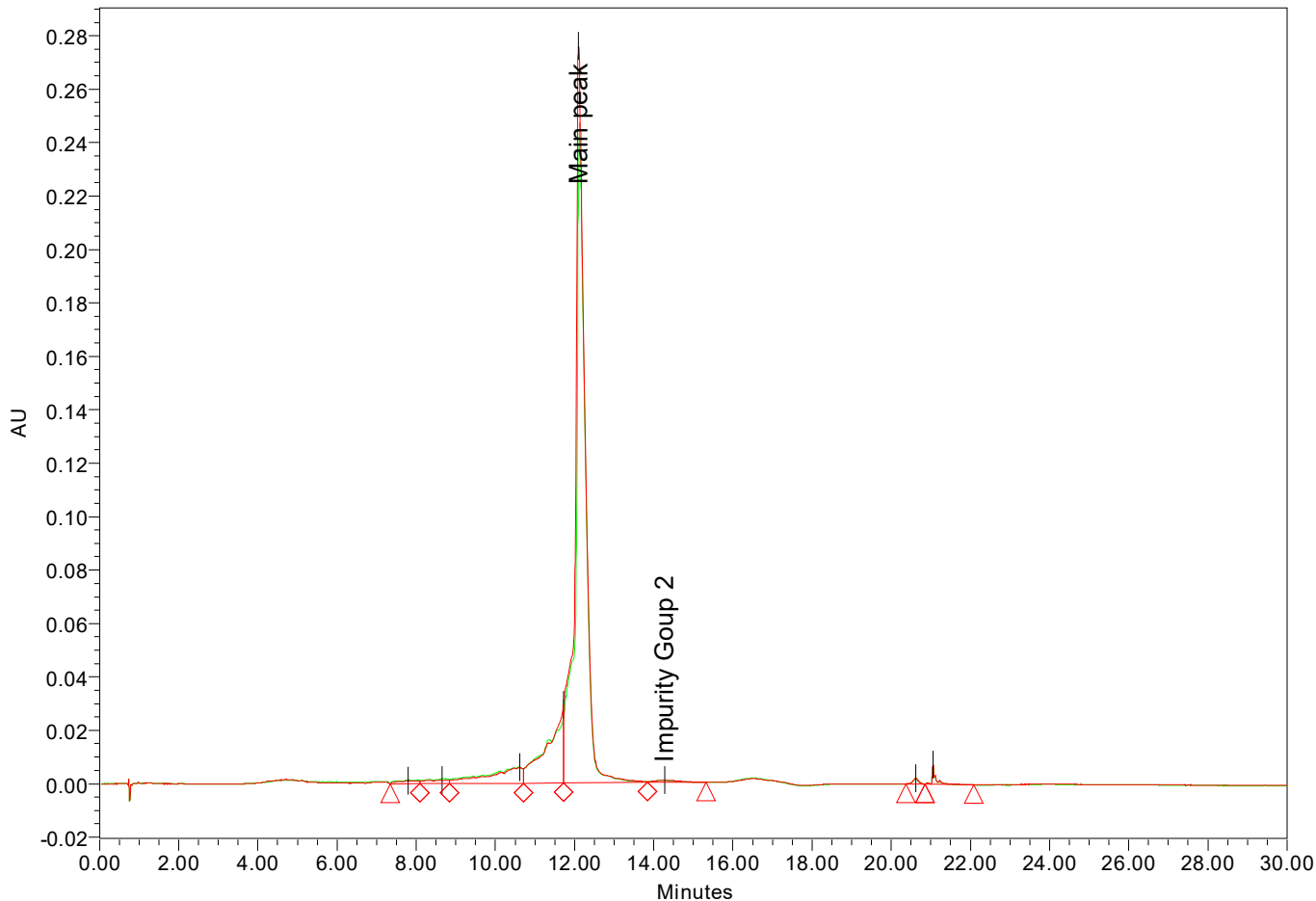
	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity Goup 2	Impurity group 3
1	U3	s47						
2	U3							
Mean								
Std. Dev.								
% RSD								

TA\_Ratio Summarized by Name  
Label: U4

	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity Goup 2	Impurity group 3
1	U4	Pfizer-P2	1	8505194	113.46	113.46		113.46
2	U4	Pfizer-P1	1	8126342	108.41	108.41		108.41
Mean				8315768				
Std. Dev.				267889				
% RSD				3.2				

# Sample Results

Sample 1



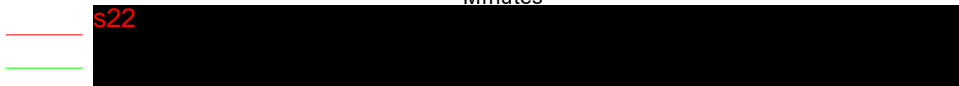
— SampleName: 2111004293-P1; Vial: 1:A,4; Injection: 1; Label U1  
 — SampleName: 2111004293-P2; Vial: 1:A,5; Injection: 1; Label U1

% Area Summarized by Name  
 Label: U1

	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity Goup 2	Impurity group 3
1	U1	2111004293-P1	1	s47				
2	U1	2111004293-P2	1					
Mean								
Std. Dev.								
% RSD								



Minutes

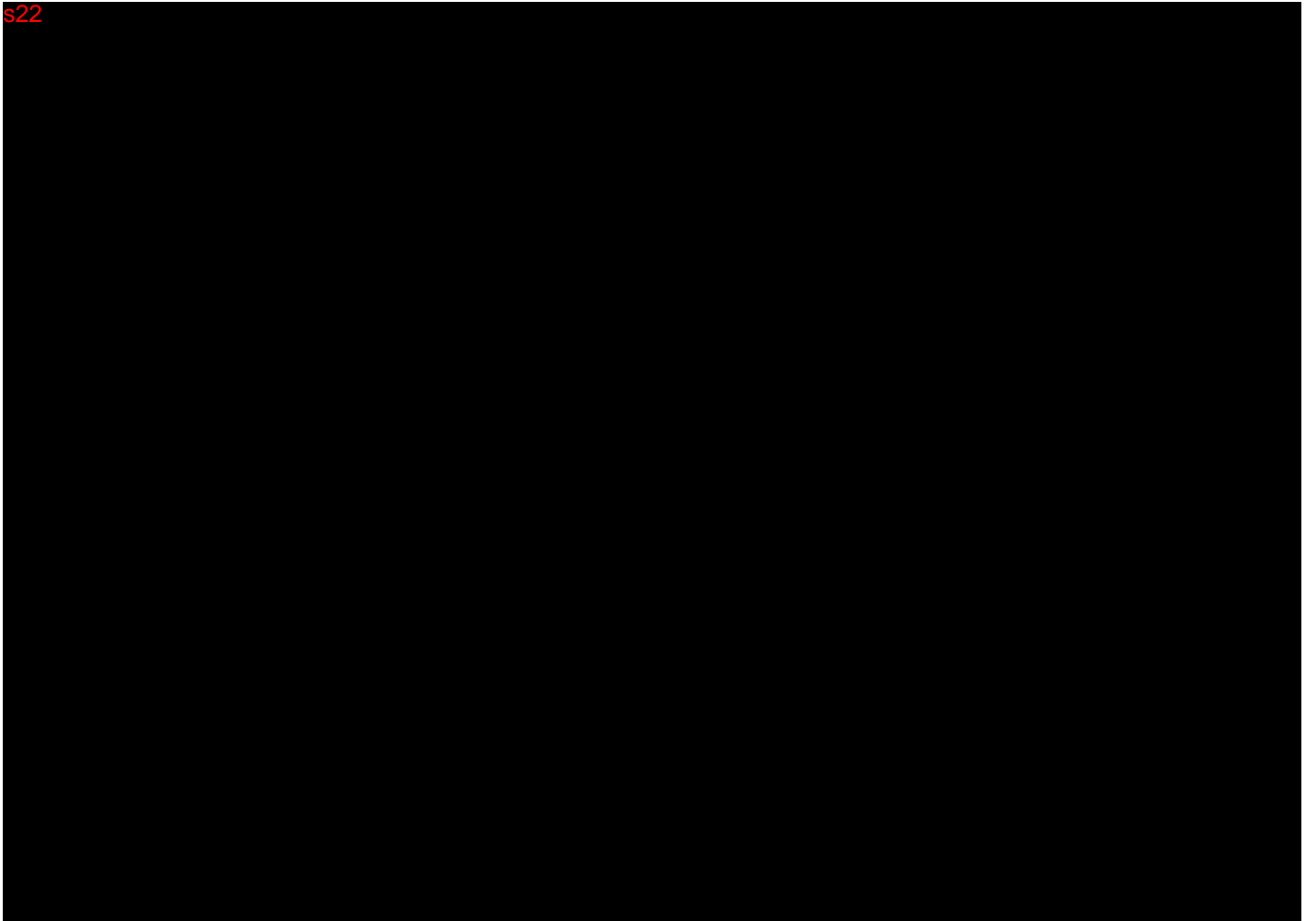


% Area Summarized by Name  
Label: U2

	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity Goup 2	Impurity group 3
1	U2	s22						
2	U2							
Mean								
Std. Dev.								
% RSD								



s22

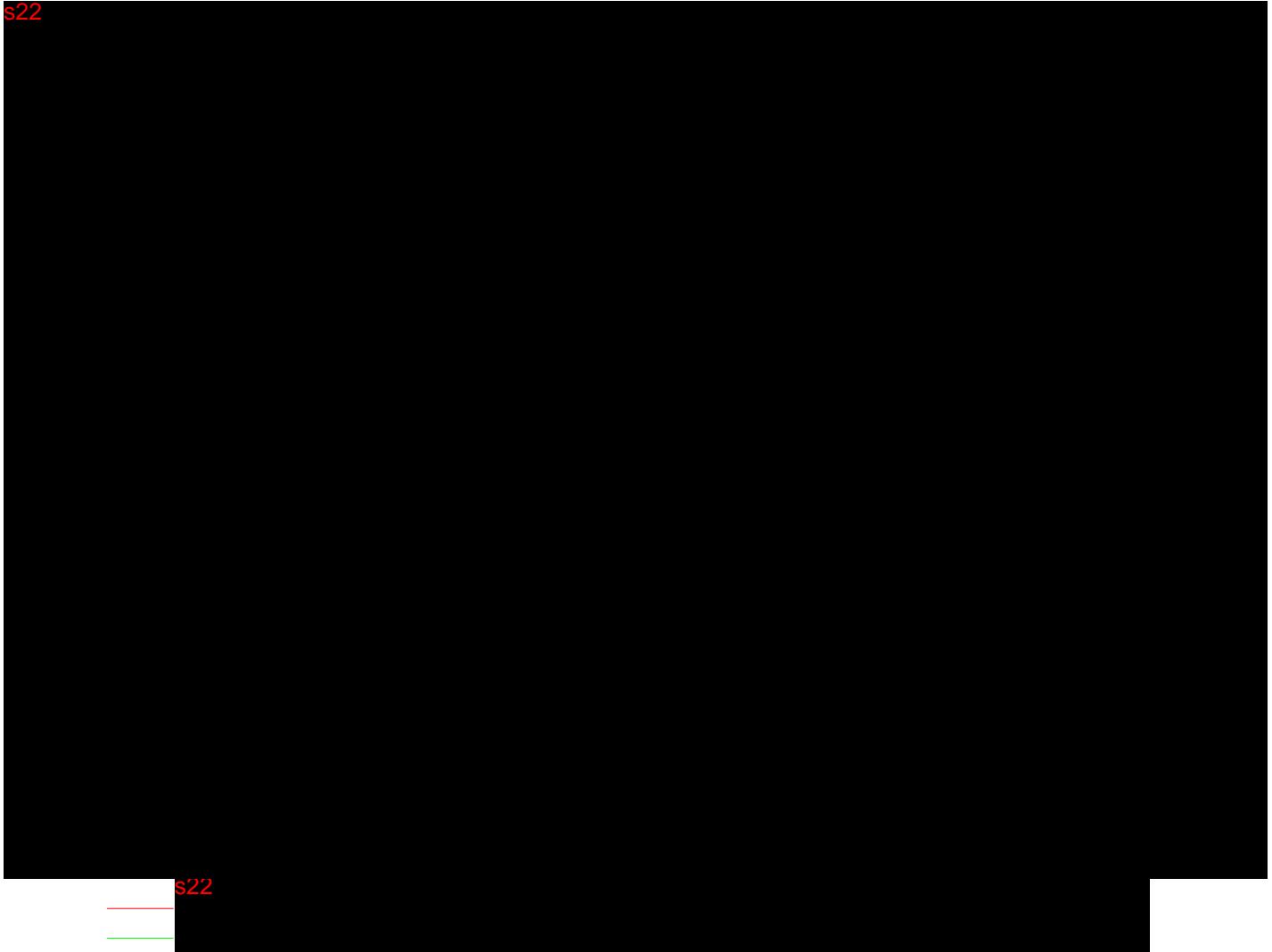


s22

% Area Summarized by Name  
Label: U3

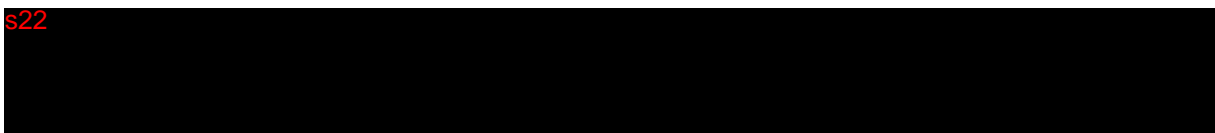
	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity Goup 2	Impurity group 3
1	U3	s22						
2	U3							
Mean								
Std. Dev.								
% RSD								

s22



% Area Summarized by Name  
Label: U4

	Label	SampleName	Inj	Total Area	Impurity group 1	Main peak	Impurity Goup 2	Impurity group 3
1	U4	s22						
2	U4							
Mean								
Std. Dev.								
% RSD								






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Error Log

Overlay Chromatogram group contains information that doesn't match the data being reported.

Amount Component Summary group contains information that doesn't match the data being reported.

	<p><b>Australian Government</b></p> <hr/> <p><b>Department of Health</b> Therapeutic Goods Administration</p>	<p>Laboratories Branch</p>
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Operations: HPLC Manual	
Procedure	HPLC – 01 – General HPLC – WORKSHEET
Written	s22
Authorised	
Date issued	12/4/2019
Revision #	8

### HPLC – 01 – General HPLC – WORKSHEET

TEST DETAILS			
TEST NAME	Analysis of mRNA purity by Size-based RPIP HPLC		
METHOD REFERENCE	Number: SOP-0996 Version: 2.0 Effective Date: 08 Apr 2021		
METHOD MODIFICATIONS (if any)	Waters Acquity system was used, instead of Thermo Vanquish system		
MODIFICATIONS APPROVED BY:	s22		
NAME OF ANALYST	s22	TEST DATE	30-Nov-2021

BUFFERS AND SOLUTIONS	
SOLUTIONS	BATCH No:
MOBILE PHASE A	SW29NOV21-1
MOBILE PHASE B	SW29NOV21-2
SAMPLE DILUENT	JG23Nov 21-1

PIPETTES USED AND EXPIRY DATES
32833 - Exp: 20/1/22 32891 Exp: 22/3/22 32892 Exp: 22/02/22 32837 Exp: 11/1/22

REFERENCE MATERIALS	
NAME AND CODE	BATCH NO:
mRNA-1273 (2019-nCoV) 2107002766	DH-03180.1

REFERENCE MATERIAL PREPARATIONS AND CALCULATIONS
Reference material was prepared as per SOP

SYSTEM SUITABILITY CRITERIA AND RESULTS			
PARAMETERS	LIMITS	RESULTS	COMMENTS
No peak in the diluent injection (blank) prior to Sensitivity Solution	No peak within assessment window	No peak	Pass
Bracketing blanks must not have interfering peaks more than 1% area of average of reference standards	Interfering peak must be $\leq 1\%$ mean peak area in SST	0.89% 0.78%	Manually integrated. refer to D21-3394260. Pass
Signal to noise ratio of Sensitivity Solution	$>10$	75	Pass
%RSD of main peak area in first five injections of reference standard	$\leq 5\%$	1.7%	Pass
%RSD of main peak retention time in first five injections of reference standard	$\leq 5\%$	0.3%	Pass
% Recovery of main peak area in bracketing standard(s) compared to average peak area of first five injections of reference standard	95-105%	98.2%	Pass
%Agreement of retention time of main peak from bracketing standard(s) compared to average Retention time of first five injections of reference standard	95-105%	99.0%	Pass
Absolute Difference of main peak area % for duplicate sample preparations	$<5\%$	2.3% <u>15.1%</u> 1.5%	sample 4294 has failed
Total Sample peak area % recovery for each replicate when compared to average total peak area of first five injections of reference standard	70-130%	82.3; 74.2% 92.8; 98.5.8 98.2; 98.7%	Pass

SAMPLE DETAILS			
SAMPLE NAME	SPIKEVAX elasomeran 0.2 mg/mL suspension for injection vial		
LIMS No:	2111004293		
BATCH No:	000098A	EXPIRY:	

**SAMPLE DILUTIONS, CALCULATIONS and DATA**

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
0.2mg/mL	-	-	0.5mg/mL	-	10µL

Prepared according to the SOP

**DATA LOCATIONS**

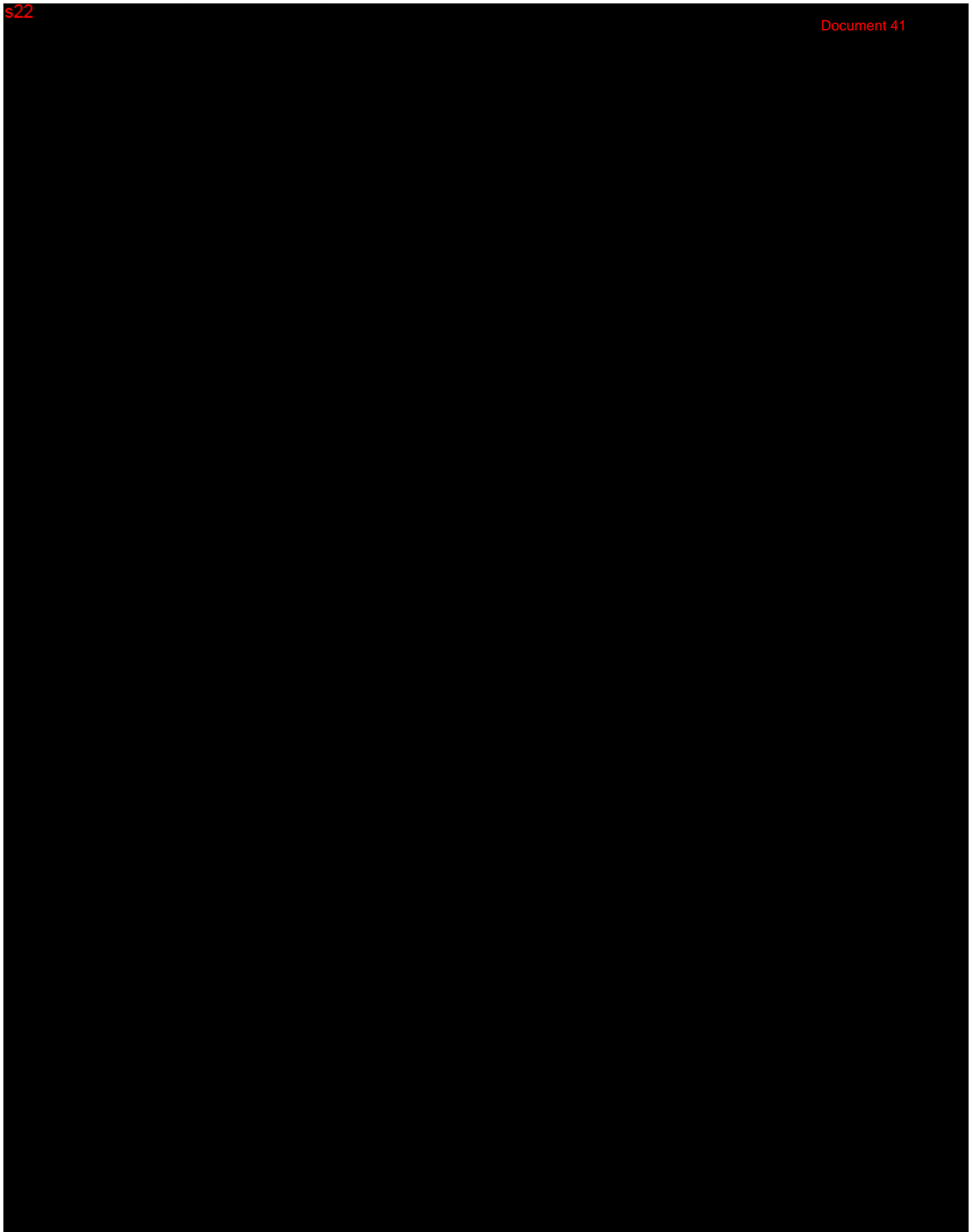
Copies of Empower reports attached?

Data location in TRIM

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS
Impurity Group 1	s47		
Main Peak Area			
Impurity Group 2			
Impurity Group 3			
Total impurities			

SAMPLE RESULTS: Pass

results in above table to be redacted

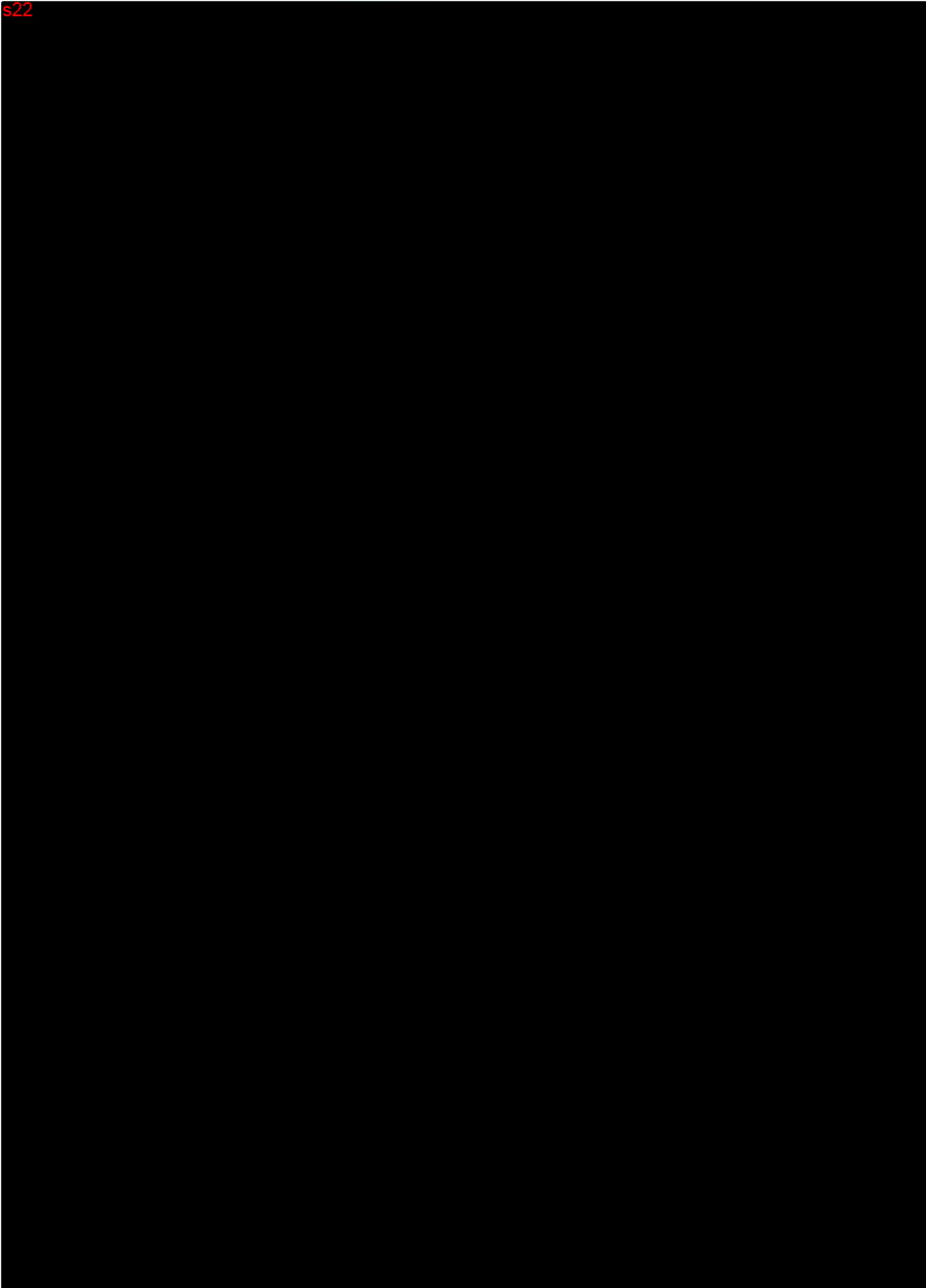


Record Details

D19-5075952 - WORKSHEET - HPLC - 01 - General HPLC

**UNCONTROLLED IF PRINTED**

S22



INTERNAL USE ONLY

IN CONFIDENCE

SAMPLE DETAILS			
SAMPLE NAME	SPIKEVAX elasomeran 0.2 mg/mL suspension for injection vial		
LIMS No:			
BATCH No:		EXPIRY:	

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
0.2mg/mL			0.5mg/mL		10
DATA LOCATIONS					
Copies of Empower reports attached?		Data location in TRIM			

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS
Impurity group 1	<sup>S47</sup> [REDACTED]	%	
Main peak	[REDACTED]	%	
Impurity group 2	[REDACTED]	%	
Impurity group 2	[REDACTED]	%	
Total impurities	[REDACTED]	%	

SAMPLE RESULTS:



**Comments**



Sample Set Summary Report

Sample Set: 30Nov21 3Moderna 1 Pfizer

Sample Set Information

Project Name: Biochemistry\2021\Moderna RP-IP HPLC

Sample Set Name: 30Nov21 3Moderna 1 Pfizer

Sample Set Acquired By: s22

Start Date: 30/11/2021 1:50:14 PM AEDT

Finish Date: 1/12/2021 6:24:48 AM AEDT

Run Time: 30.00 Minutes

Sample Set Altered: Yes

SampleName: 2111004294-P2, Pfizer-P1, Water Blank, Reference Standard, 2111004294-P1, 2111004295-P1, 2111004293-P1, water, 2111004293-P2, 2111004295-P2, Conditioning, Sensitivity Solution, 2111004293, Pfizer-P2

Acq Method Set: Moderna RP IP

Instrument Method Name: Moderna RP IP

Sample Set Method: Moderna RP IP SSM v2

System Information

System Name: System 4 PDA Only

Empower Node: Ucdp191prk2

Analytical\_Column\_1: ID# 500 IonPac AS11-HC

Analytical\_Column\_2:

Processing Information

Processing Method: Moderna RP IP\_HPLC\_10min

Processed By: s22/Biochem

Processing Method Id: 8068

Date Processed: 1/12/2021 5:11:53 PM AEDT,  
1/12/2021 5:11:54 PM AEDT, 1/12/2021 5:11:55 PM AEDT  
Processing Node: Uclpdbnjin13

Result Set Name: 30Nov21 3Moderna 1 Pfizer

Result Set Date: 1/12/2021 5:11:53 PM AEDT

Result Set Id: 8071

Channel Description: PDA Ch1 260nm@4.8nm

Reporting Information

Report Method Name: Sample Set Summary Report

Reported by: s22 )

Print Date: 2/12/2021

Time: 11:57:55 AM Australia/Canberra

## Injection Sequence Summary

	SampleName	Sample Type	Vial	Inj #	Run Time (Minutes)	Injection Volume (ul)	Sample Weight	Dilution	Level	Label
1	water	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		
2	Conditioning	Control	1:A,3	1	30.00	10.00	1.00000	1.00000		
3	Conditioning	Control	1:A,3	2	30.00	10.00	1.00000	1.00000		
4	Conditioning	Control	1:A,3	3	30.00	10.00	1.00000	1.00000		
5	Conditioning	Control	1:A,3	4	30.00	10.00	1.00000	1.00000		
6	Conditioning	Control	1:A,3	5	30.00	10.00	1.00000	1.00000		
7	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B11
8	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B12
9	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B13
10	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B14
11	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B2
12	Sensitivity Solution	Standard	1:A,2	1	30.00	10.00	1.00000	1.00000		S
13	Conditioning	Control	1:A,3	1	30.00	10.00	1.00000	1.00000		
14	Reference Standard	Standard	1:A,3	1	30.00	10.00	1.00000	1.00000		R1
15	Reference Standard	Standard	1:A,3	2	30.00	10.00	1.00000	1.00000		R1
16	Reference Standard	Standard	1:A,3	3	30.00	10.00	1.00000	1.00000		R1
17	Reference Standard	Standard	1:A,3	4	30.00	10.00	1.00000	1.00000		R1
18	Reference Standard	Standard	1:A,3	5	30.00	10.00	1.00000	1.00000		R1
19	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B3
20	2111004293	Control	1:A,4	1	30.00	10.00	1.00000	1.00000		
21	2111004293-P1	Unknown	1:A,4	1	30.00	10.00	1.00000	1.00000		U1
22	2111004293-P2	Unknown	1:A,5	1	30.00	10.00	1.00000	1.00000		U1
23	2111004294-P1	Unknown	1:A,6	1	30.00	10.00	1.00000	1.00000		U2
24	2111004294-P2	Unknown	1:A,7	1	30.00	10.00	1.00000	1.00000		U2
25	2111004295-P1	Unknown	1:B,1	1	30.00	10.00	1.00000	1.00000		U3
26	2111004295-P2	Unknown	1:B,2	1	30.00	10.00	1.00000	1.00000		U3
27	Pfizer-P1	Unknown	1:B,3	1	30.00	10.00	1.00000	1.00000		U4
28	Pfizer-P2	Unknown	1:B,4	1	30.00	10.00	1.00000	1.00000		U4
29	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B4
30	Conditioning	Control	1:A,3	1	30.00	10.00	1.00000	1.00000		
31	Reference Standard	Standard	1:A,3	1	30.00	10.00	1.00000	1.00000		R3
32	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B5



Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

Owner: s22	Number: Bio-BEE-Form-42
Author: s22	Version: 1
Active: 2/07/2021	Review: <QPulse_DocReviewDate>
Title: Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 23Nov2021

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899

LRW Expiry: 18 March 2022

Other Reagent: Pyrospense Batch# 0000904583  
December 2021

Expiry: 29 June 2022 Use By: 21

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Moderna</u>	<u>000098A</u>	<u>25 May 2022</u>	<u>2111004293</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
<u>n/a</u>	s47	<u>2000</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of vaccine</u>	<u>975uL</u>	<u>5uL Pyrospense</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrospense</u>
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>N/A (undefined)</u>	s47	<u>0.17</u>	<u>104</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

### Notes:

**Sample appears translucent, white solution, very homogeneous and free from particulates**

**Checked** s22 **24Nov2021**



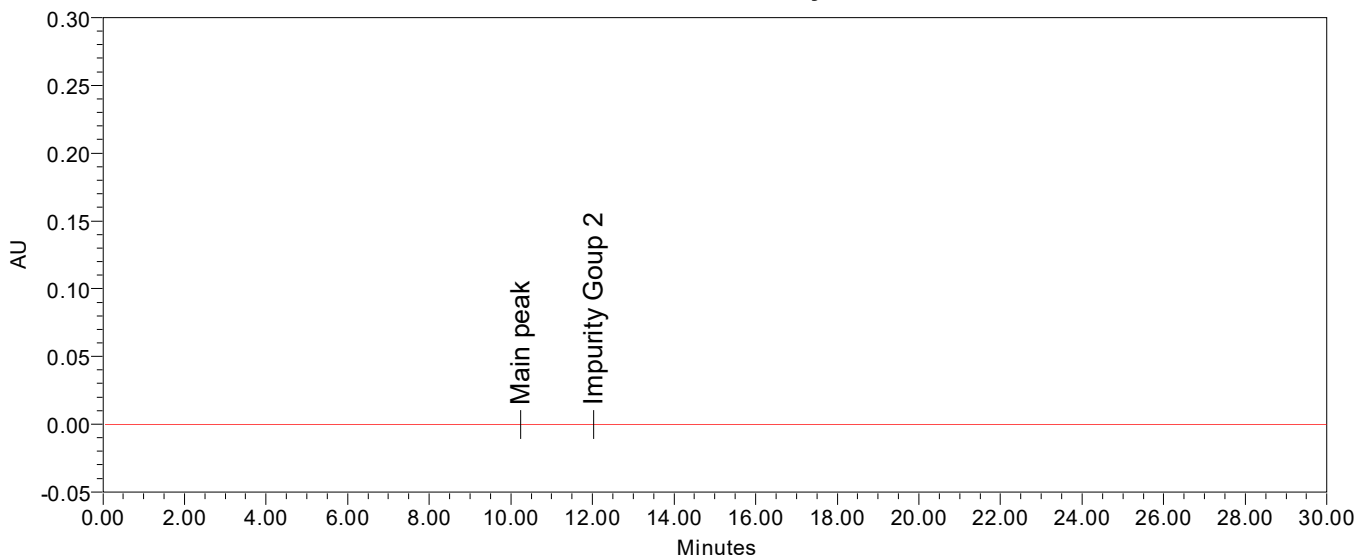
Australian Government  
Department of Health and Ageing  
Therapeutic Goods Administration

Water Blank. Reference Standard. 2109003420-P2,  
s22

Sample Set Name: Spikevax\_IEX\_23Sep21\_TK  
Sample Set Acquired By: s22

## System Suitability

Water blank - Prior to sensitivity standard

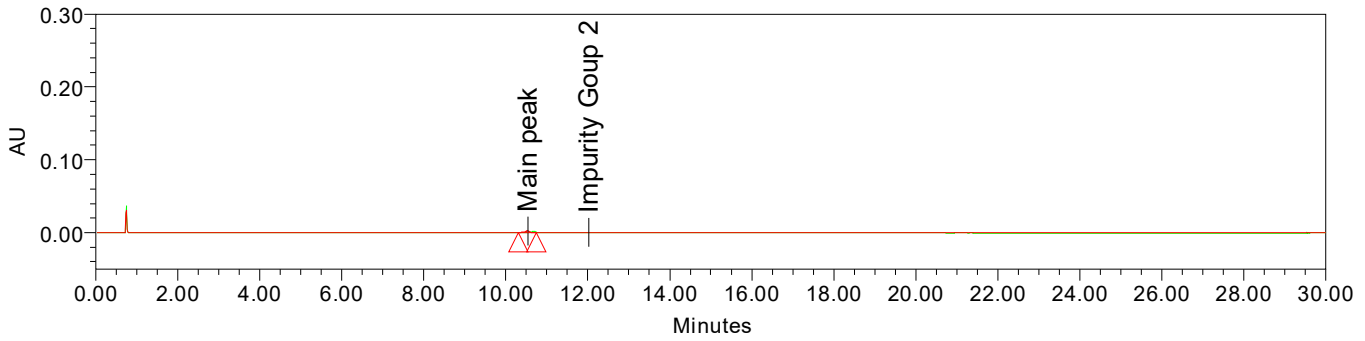


— Label B2; SampleName: Water Blank; Vial: 1:A,1; Injection: 1

Component Results

	Name	RT	Area	Height	Amount	Units	Total Area	TA_Ratio
1	Impurity Group 3						14645	
2	Impurity Group 1						14645	
3	Main peak	10.535	14645	2120			14645	0.13
4	Impurity Goup 2	12.022					14645	

Water blanks - carryover assessment



— Label B3; SampleName: Water Blank; Injection: 1; Channel PDA Ch1 260nm@4.8nm  
 — Label B4; SampleName: Water Blank; Injection: 1; Channel PDA Ch1 260nm@4.8nm

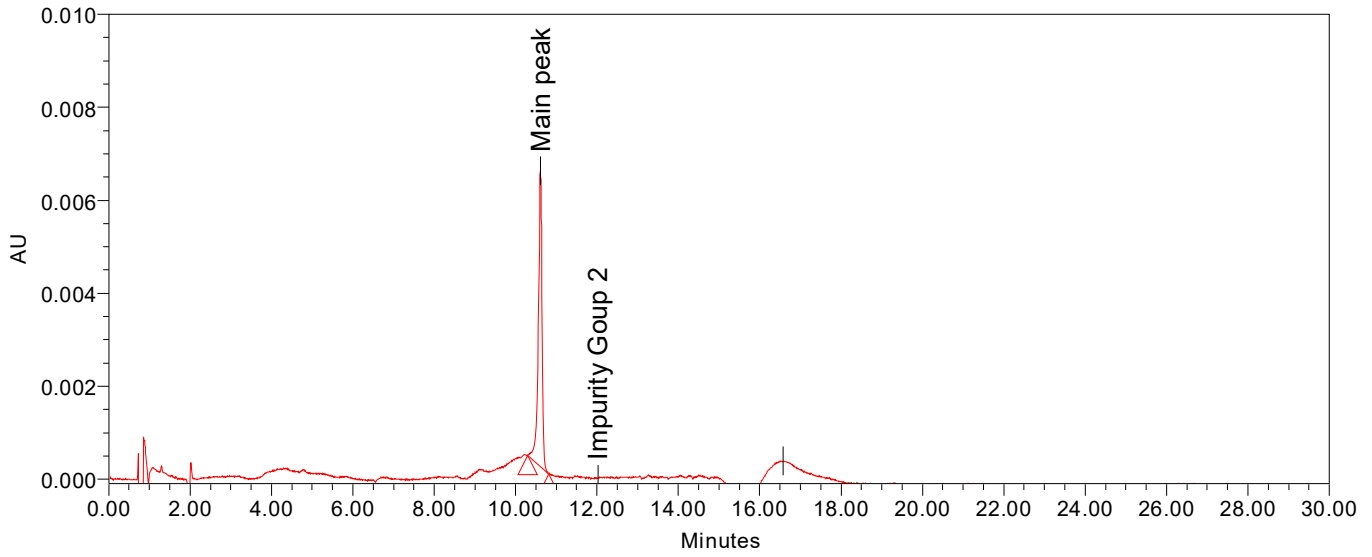
TA\_Ratio Summarized by Name  
 Label: B3

	Label	SampleName	Inj	Total Area	Main peak
1	B3	Water Blank	1	14645	0.13
Mean				14645	
Std. Dev.					
% RSD					

TA\_Ratio Summarized by Name  
 Label: B4

	Label	SampleName	Inj	Total Area	Main peak
1	B4	Water Blank	1	13019	0.11
Mean				13019	
Std. Dev.					
% RSD					

Sensitivity Solution



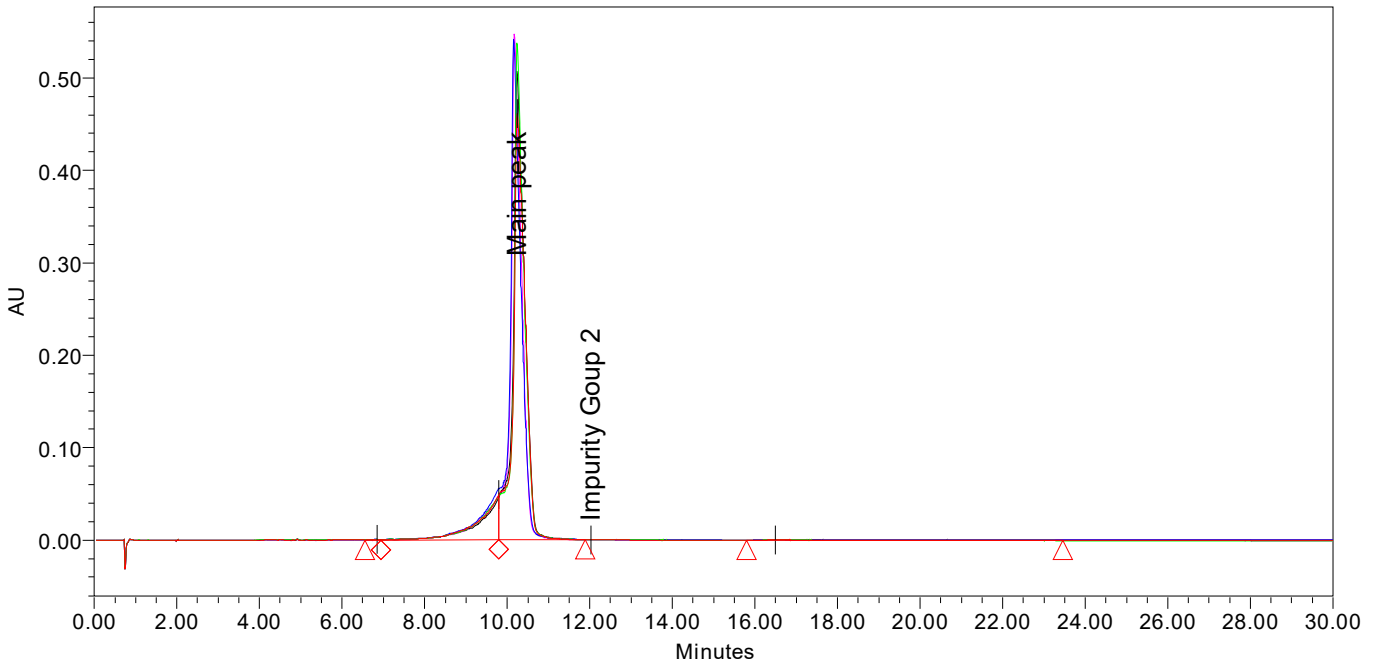
SampleName: Sensitivity Solution; Vial: 1:A,2; Injection: 1; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm

Sensitivity Solution

	Name	RT	USP Tailing	USP s/n	Area (μV*sec)	EP s/n	Total Area	TA_Ratio
1	Main peak	10.609	0.876863	376.7	42195	376.7	149624	1.31



First five reference standard injections

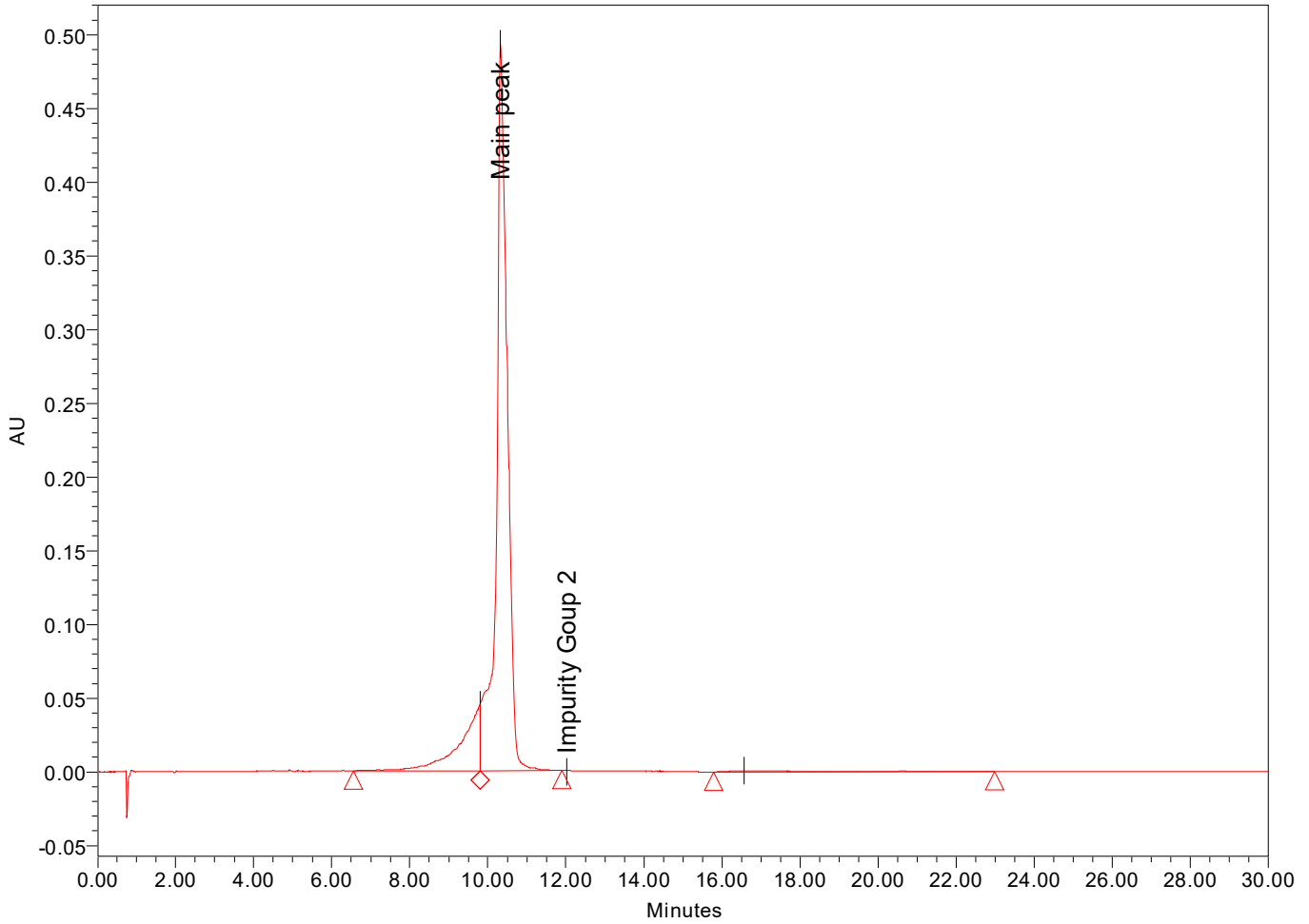


- SampleName: Reference Standard; Vial: 1:A,3; Injection: 1; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 2; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 3; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 4; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 5; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm

Component Summary Table  
Name: Main peak

	Sample Name	Vial	Inj	Channel	RT	Area	Total Area
1	Reference Standard	1:A,3	1	PDA Ch1 260nm@4.8nm	10.240	9270280	10996962
2	Reference Standard	1:A,3	2	PDA Ch1 260nm@4.8nm	10.237	9601303	11247027
3	Reference Standard	1:A,3	3	PDA Ch1 260nm@4.8nm	10.157	9664763	11598434
4	Reference Standard	1:A,3	4	PDA Ch1 260nm@4.8nm	10.231	9952794	11566276
5	Reference Standard	1:A,3	5	PDA Ch1 260nm@4.8nm	10.172	9794947	11577562
Mean					10.207	9656817	11397252
Std. Dev.					0.039	254577	266624.6
% RSD					0.4	2.6	2.3

Bracketing Reference Standard



— SampleName: Reference Standard; Vial: 1:A,3; Injection: 1; Label R3

Component Summary Table  
Name: Main peak

	Sample Name	Vial	Inj	Channel	RT	Area	Total Area	Area_Ratio	RT_Ratio
1	Reference Standard	1:A,3	1	PDA Ch1 260nm@4.8nm	10.321	9557030	11251534	99.0	101.1
Mean					10.321	9557030	11251534		
Std. Dev.									
% RSD									

## Sample Acceptance Criteria

TA\_Ratio Summarized by Name

Label: U1

	Label	SampleName	Inj	Total Area	Impurity Group 1	Main peak	Impurity Goup 2	Impurity Group 3
1	s22							
2								
Mean								
Std. Dev.								
% RSD								

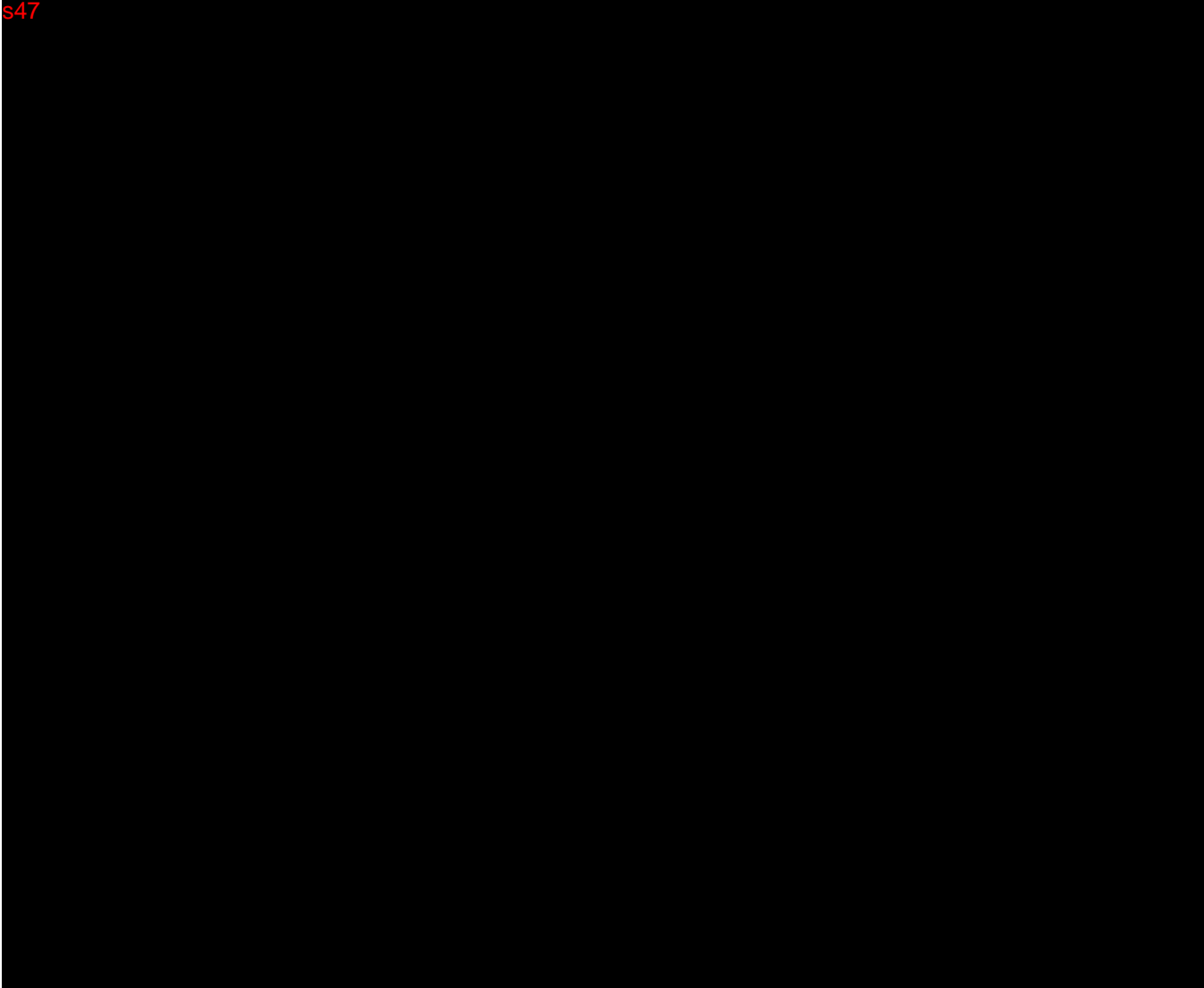
TA\_Ratio Summarized by Name

Label: U2

	Label	SampleName	Inj	Total Area	Impurity Group 1	Main peak	Impurity Goup 2	Impurity Group 3
1	U2	s47						
2	U2							
Mean								
Std. Dev.								
% RSD								

### Sample Results

s47

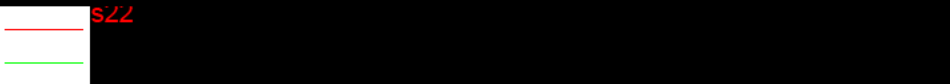
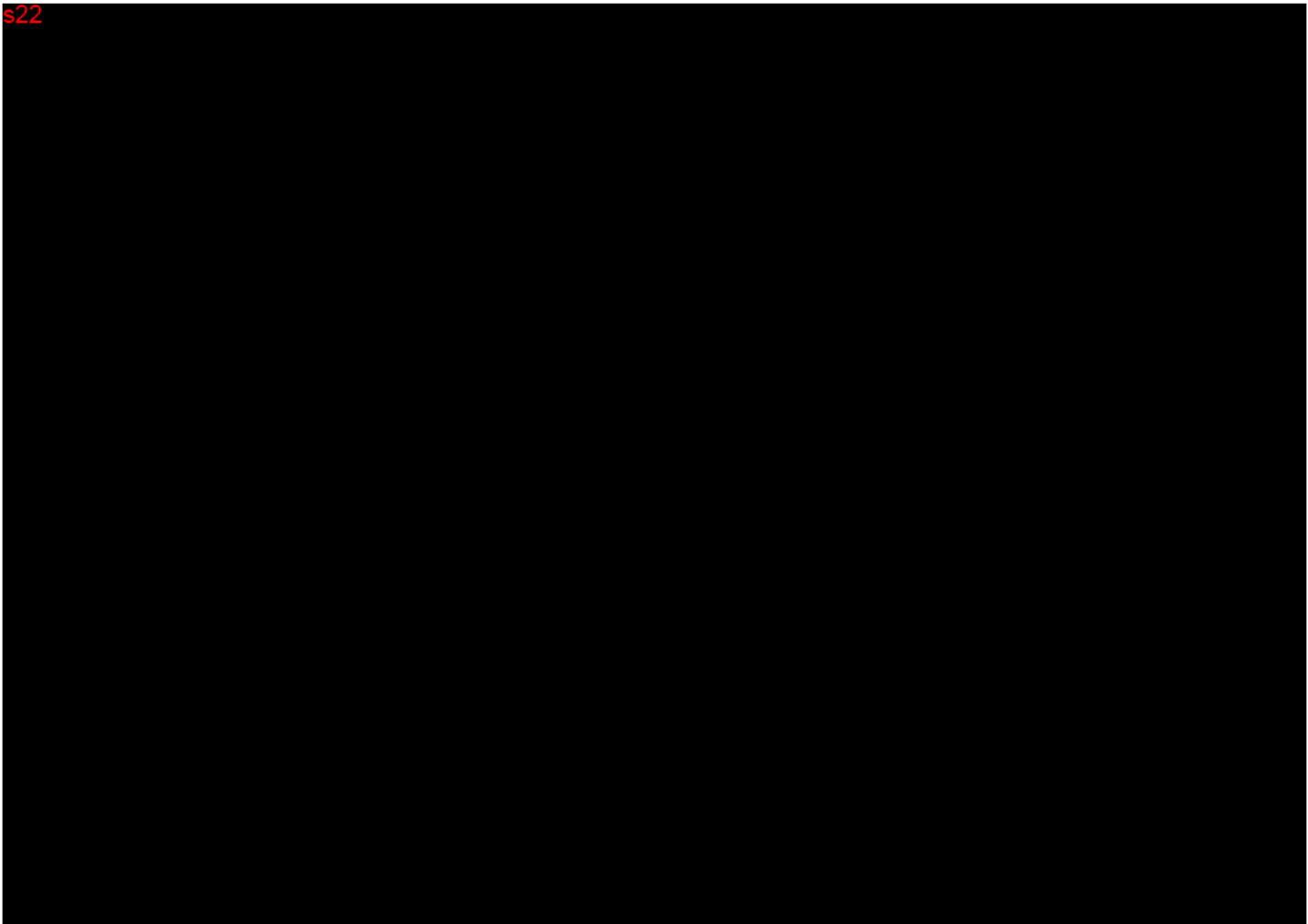


% Area Summarized by Name  
Label: U1

	Label	SampleName	Inj	Total Area	Impurity Group 1	Main peak	Impurity Goup 2	Impurity Group 3
1	U1	2109003420-P1	1	s47				
2	U1	2109003420-P2	1					
Mean								
Std. Dev.								
% RSD								

s22





% Area Summarized by Name  
Label: U2

	Label	SampleName	Inj	Total Area	Impurity Group 1	Main peak	Impurity Goup 2	Impurity Group 3
1	U2	s22						
2	U2							
Mean								
Std. Dev.								
% RSD								





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Error Log

Overlay Chromatogram group contains information that doesn't match the data being reported.  
Amount Component Summary group contains information that doesn't match the data being reported.  
Overlay Chromatogram group contains information that doesn't match the data being reported.  
Amount Component Summary group contains information that doesn't match the data being reported.  
Overlay Chromatogram group contains information that doesn't match the data being reported.  
Amount Component Summary group contains information that doesn't match the data being reported.





**Australian Government**  
**Department of Health**  
**Therapeutic Goods Administration**

Laboratories Branch

Operations: HPLC Manual

<b>Procedure</b>	HPLC – 01 – General HPLC – WORKSHEET
<b>Written</b>	s22
<b>Authorised</b>	
<b>Date issued</b>	12/4/2019
<b>Revision #</b>	8

**HPLC – 01 – General HPLC – WORKSHEET**

<b>TEST DETAILS</b>			
<b>TEST NAME</b>	Analysis of mRNA purity by Size-based RPIP HPLC		
<b>METHOD REFERENCE</b>	Number: SOP-0996 Version: 2.0 Effective Date: 08 Apr 2021		
<b>METHOD MODIFICATIONS (if any)</b>	Waters Acquity system was used, instead of Thermo Vanquish system		
<b>MODIFICATIONS APPROVED BY:</b>	s22		
<b>NAME OF ANALYST</b>	s22	<b>TEST DATE</b>	23-Sep-2021

<b>BUFFERS AND SOLUTIONS</b>	
<b>SOLUTIONS</b>	<b>BATCH No:</b>
MOBILE PHASE A	SW-Mob Ph A-13Sep21
MOBILE PHASE B	SW-Mob Ph B-21Sep21
SAMPLE DILUENT	SW-Dil -13Sep21

Record Details

D19-5386724 - WORKSHEET - HPLC – 01 - General HPLC

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Page 1 of 8

PIPETTES USED AND EXPIRY DATES
32833 - Exp: 28/10/21 32891 Exp: 10/12/21 32892 Exp: 24/11/21 32837 Exp: 24/11/21

REFERENCE MATERIALS	
NAME AND CODE	BATCH NO:
mRNA-1273 (2019-nCoV) 2107002766	DH-03180.1

REFERENCE MATERIAL PREPARATIONS AND CALCULATIONS
Reference material was prepared as per SOP

INTERNAL USE ONLY

IN CONFIDENCE

SYSTEM SUITABILITY CRITERIA AND RESULTS			
PARAMETERS	LIMITS	RESULTS	COMMENTS
No peak in the diluent injection (blank) prior to Sensitivity Solution	No peak within assessment window	No peak	Pass
Bracketing blanks must not have interfering peaks more than 1% area of average of reference standards	Interfering peak must be $\leq 1\%$ mean peak area in SST	0.13% 0.11%	Pass
Signal to noise ratio of Sensitivity Solution	$>10$	376	Pass
%RSD of main peak area in first five injections of reference standard	$\leq 5\%$	2.6%	Pass
%RSD of main peak retention time in first five injections of reference standard	$\leq 5\%$	0.4%	Pass
% Recovery of main peak area in bracketing standard(s) compared to average peak area of first five injections of reference standard	95-105%	99.0%	Pass
%Agreement of retention time of main peak from bracketing standard(s) compared to average Retention time of first five injections of reference standard	95-105%	101.1%	Pass
Absolute Difference of main peak area % for duplicate sample preparations	$<5\%$	1.0% 1.6%	Pass
Total Sample peak area % recovery for each replicate when compared to average total peak area of first five injections of reference standard	70-130%	94.9% 94.7% 91.1% 88.1%	Pass

INTERNAL USE ONLY

IN CONFIDENCE

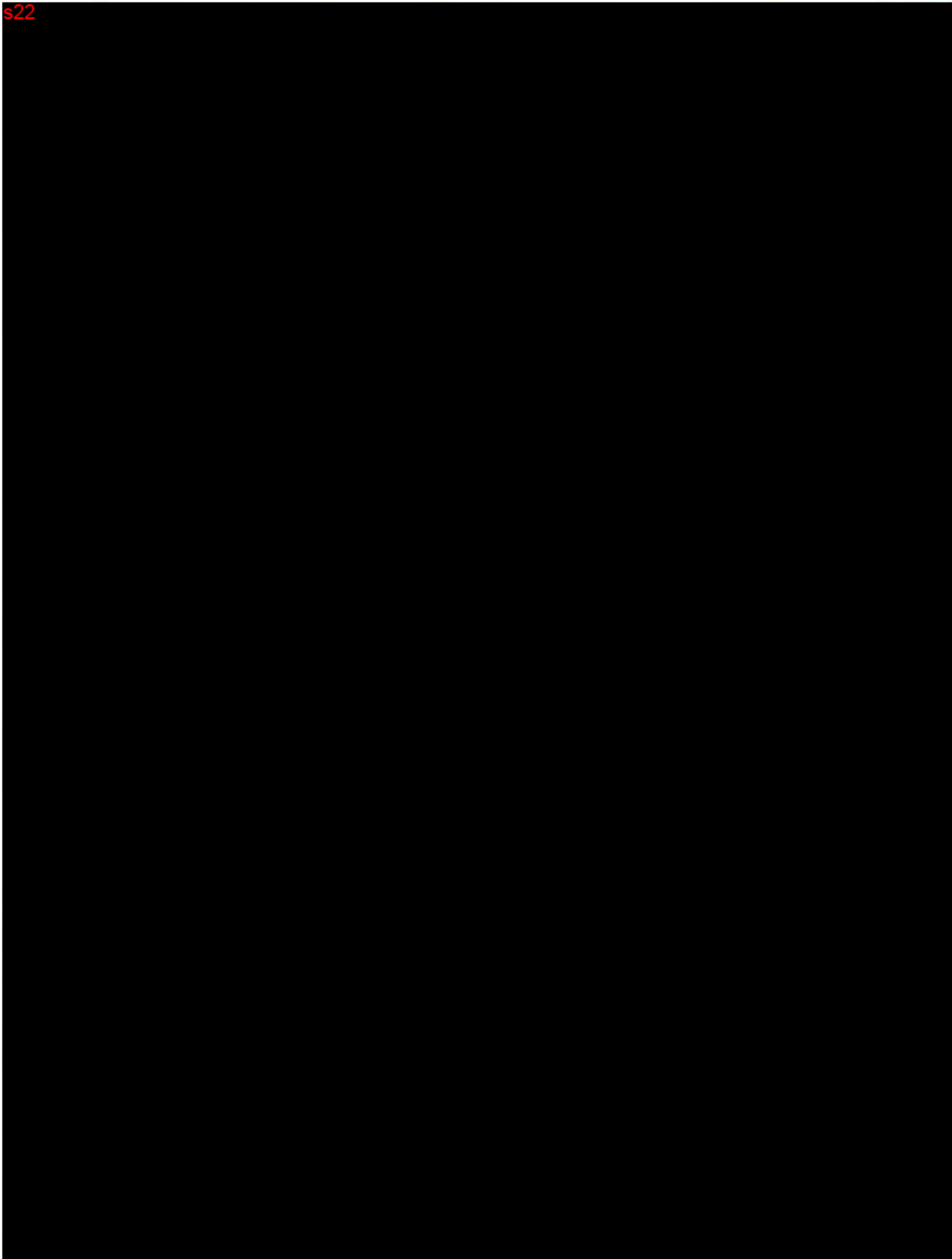
SAMPLE DETAILS			
SAMPLE NAME	SPIKEVAX elasomeran 0.2 mg/mL suspension for injection vial		
LIMS No:	2109003420-R1		
BATCH No:	000016A	EXPIRY:	26-Mar-2022

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
0.2mg/mL	-	-	0.5mg/mL	-	10µL
Prepared according to the SOP					
DATA LOCATIONS					
Copies of Empower reports attached?		Data location in TRIM			

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS
Impurity Group 1	s47		
Main Peak Area			
Impurity Group 2			
Impurity Group 3			
Total impurities			

SAMPLE RESULTS: Pass

s22



**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

DATA LOCATIONS	
Copies of Empower reports attached?	Data location in TRIM

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

DATA LOCATIONS		
Copies of Empower reports attached?		Data location in TRIM

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**Comments**





Sample Set Summary Report

Sample Set: Spikevax\_IEX\_23Sep21\_TK

Sample Set Information

Project Name: Biochemistry\2021\Moderna RP-IP HPLC

Sample Set Name: Spikevax\_IEX\_23Sep21\_TK

SampleName: Water Blank, Reference Standard,  
2109003420-P2, 2109003420-P1, Conditioning,  
2109003470-P1, Sensitivity Solution, 2109003470-P2

Sample Set Acquired By: s22

Start Date: 23/09/2021 12:16:53 PM AEST

Finish Date: 23/09/2021 11:40:39 PM AEST

Acq Method Set: Moderna RP IP

Run Time: 30.00 Minutes

Instrument Method Name: Moderna RP IP

Sample Set Altered: No

Sample Set Method: Moderna RP IP SSM v2

System Information

System Name: System 4 PDA Only

Analytical\_Column\_1: ID# 500 IonPac AS11-HC

Empower Node: Ucdp191prk2

Analytical\_Column\_2:

Processing Information

Processing Method: Moderna RP IP\_HPLC\_10min

Result Set Name: Spikevax\_IEX\_23Sep21\_TK

Processed By: s22/Biochem

Result Set Date: 24/09/2021 9:55:52 AM AEST

Processing Method Id: 3159

Result Set Id: 3160

Date Processed: 24/09/2021 9:55:53 AM AEST,  
24/09/2021 9:55:54 AM AEST, 24/09/2021 9:55:55

Channel Description: PDA Ch1 260nm@4.8nm

Processing Node: Uclpdbnjn13

Reporting Information

Report Method Name: Sample Set Summary Report

Print Date: 24/09/2021

Reported by: s22

Time: 12:15:21 PM Australia/Canberra

## Injection Sequence Summary

	SampleName	Sample Type	Vial	Inj #	Run Time (Minutes)	Injection Volume (ul)	Sample Weight	Dilution	Level	Label
1	Conditioning	Control	1:A,3	1	30.00	10.00	1.00000	1.00000		
2	Conditioning	Control	1:A,3	2	30.00	10.00	1.00000	1.00000		
3	Conditioning	Control	1:A,3	3	30.00	10.00	1.00000	1.00000		
4	Conditioning	Control	1:A,3	4	30.00	10.00	1.00000	1.00000		
5	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B11
6	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B12
7	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B13
8	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B14
9	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B2
10	Sensitivity Solution	Standard	1:A,2	1	30.00	10.00	1.00000	1.00000		S
11	Reference Standard	Standard	1:A,3	1	30.00	10.00	1.00000	1.00000		R1
12	Reference Standard	Standard	1:A,3	2	30.00	10.00	1.00000	1.00000		R1
13	Reference Standard	Standard	1:A,3	3	30.00	10.00	1.00000	1.00000		R1
14	Reference Standard	Standard	1:A,3	4	30.00	10.00	1.00000	1.00000		R1
15	Reference Standard	Standard	1:A,3	5	30.00	10.00	1.00000	1.00000		R1
16	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B3
17	2109003420-P1	Unknown	1:A,4	1	30.00	10.00	1.00000	1.00000		U1
18	2109003420-P2	Unknown	1:A,5	1	30.00	10.00	1.00000	1.00000		U1
s22										
21	Water Blank	Control	1:A,1	1	30.00	10.00	1.00000	1.00000		B4
22	Reference Standard	Control	1:A,3	1	30.00	10.00	1.00000	1.00000		R3



Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

Owner: s22	Number: Bio-BEE-Form-42
Author: s22	Version: 1
Active: 2/07/2021	Review: <QPulse_DocReviewDate>
Title: Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 21Sep2021

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899 LRW Expiry: 18 March 2022

Other Reagent: Pyrospense Batch# 0000904583 Expiry: 29 June 2022  
Use By: 19 October 2021

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Moderna covid vaccine</u>	<u>000016A</u>	<u>26 March 2022</u>	<u>2109003420-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
<u>n/a</u>	s47	<u>2000</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of vaccine</u>	<u>975uL</u>	<u>5uL Pyrospense</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrospense</u>
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>N/A (Undefined)</u>	s47	<u>1.23</u>	<u>142</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

**Notes:**

Sample appears translucent, white solution, very homogeneous and free from particulates

Pyrospense Use by date is incorrect on Endotoxin report – should be as shown above (19Oct2021)

Checked s22 22Sep2021 & 12Jan2023

## Instrument controller software run summary:

**Filename and data path:** C:\Agilent Technologies\Data\2021 10 26\13-56-20\2021 10 26 13H 56M.raw

**Created:** Tuesday, October 26, 2021 2:21:46 PM

**Number of capillaries:** 19

**Array serial number:** 022621-27SFS

**Effect length:** 33 cm

**Array usage count:** 30

**Instrument type:** 5300 Fragment Analyzer

**Instrument controller software version:** 3.1.0.12

**Device serial number:** MY2105AB19

## Method Information

**Method name:** DNF-471E33 - SS Total RNA 15nt Extended.mthds

**Gel prime:** No

**Full conditioning:** Yes

**Gel prime to buffer:** Yes

**Gel selection:** Gel 2

**Perform prerun:** 8.0 kV, 30 sec.

**Rinse:** No

**Marker 1:** No

**Rinse:** Tray: 3, Row: A, Dip count: 2

**Sample injection:** 5.0 kV, 6 sec.

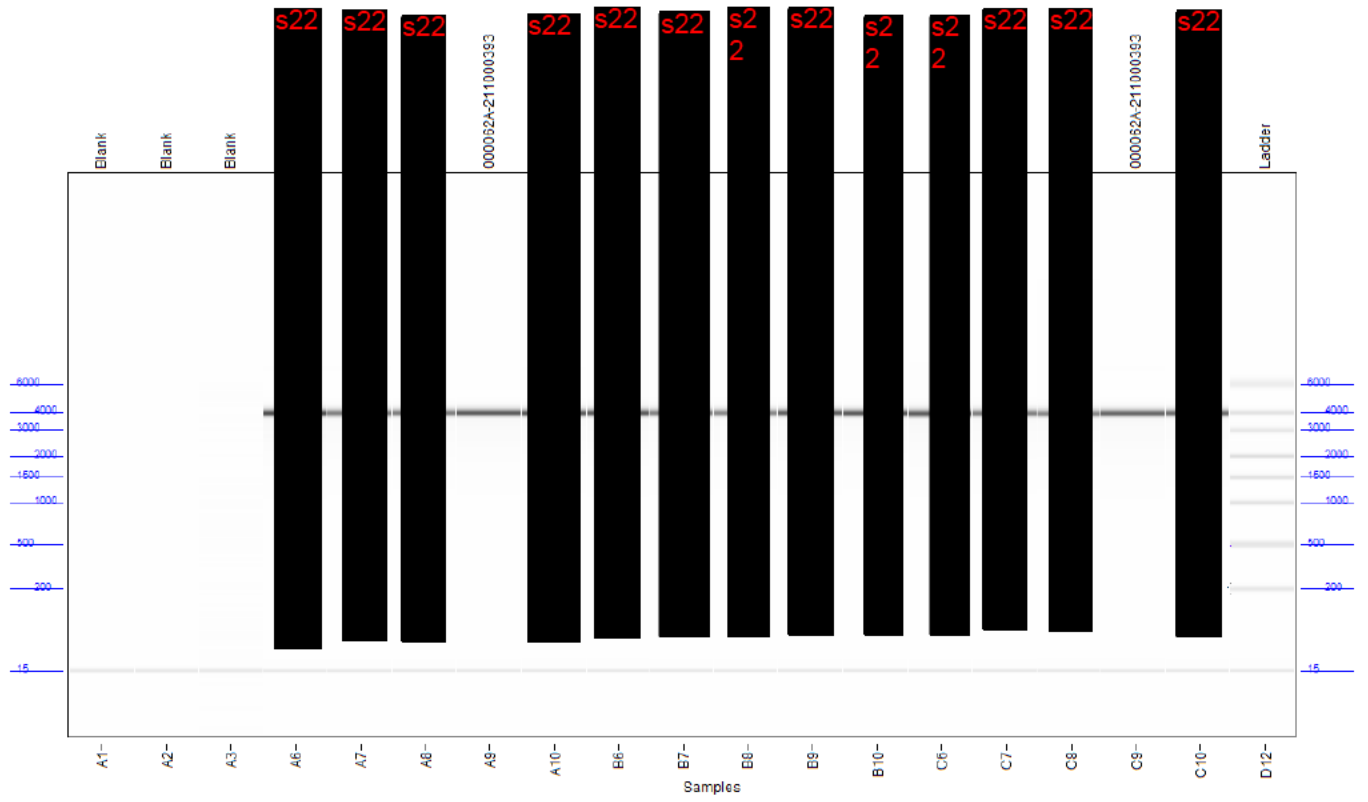
**Separation:** 8.0 kV, 60.0 min.

**Tray name:** Tray-1

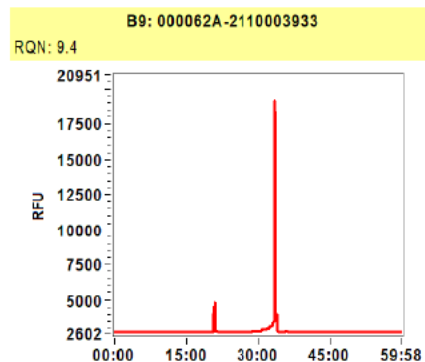
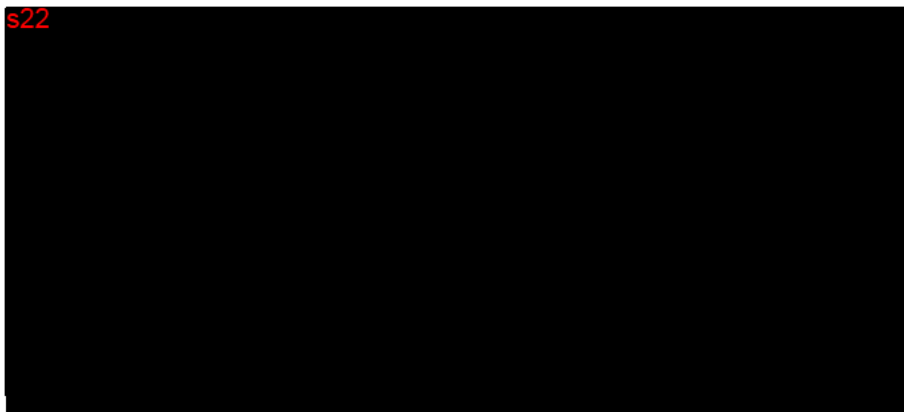
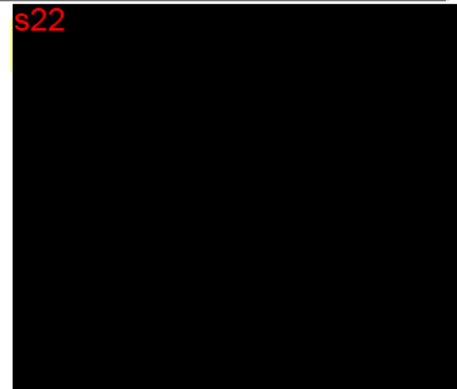
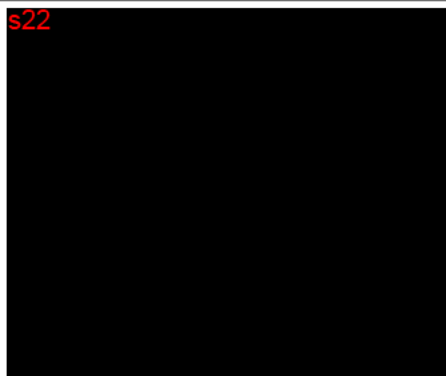
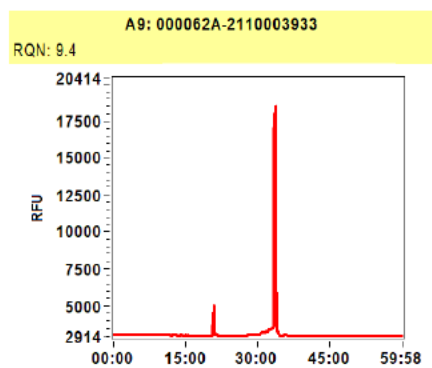
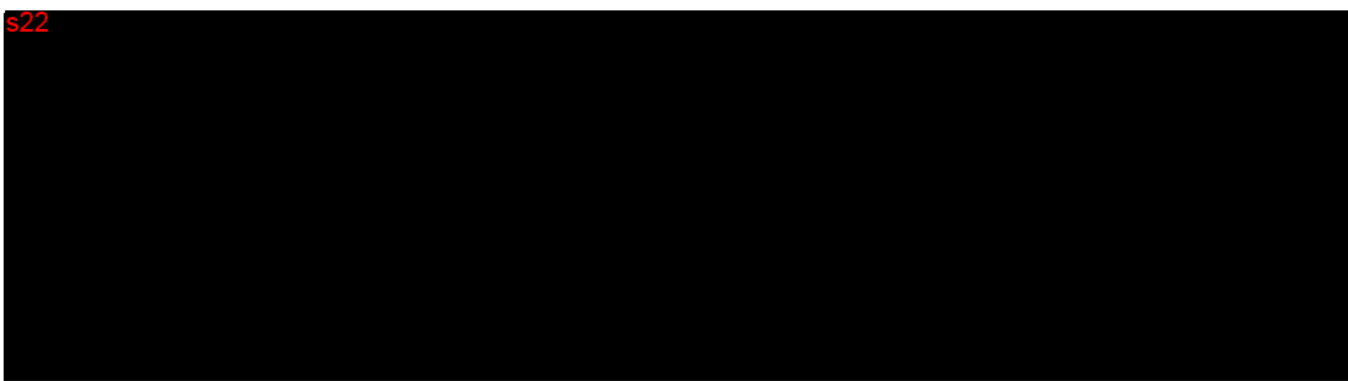
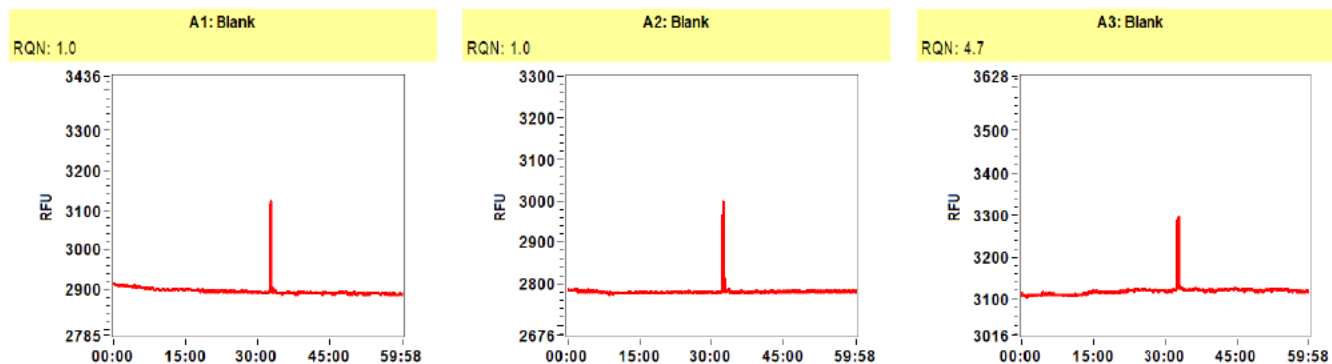
**Analysis mode:** RNA (Eukaryotic)

## Notes

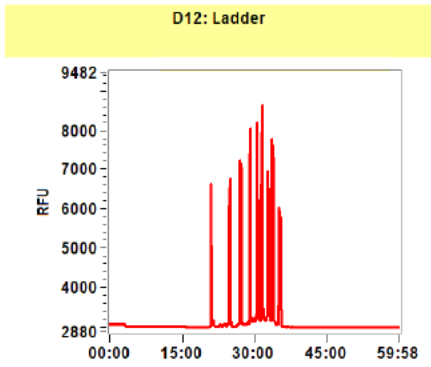
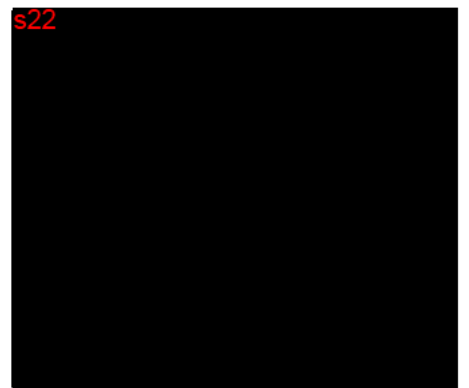
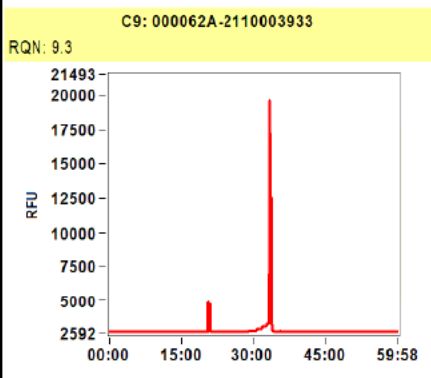
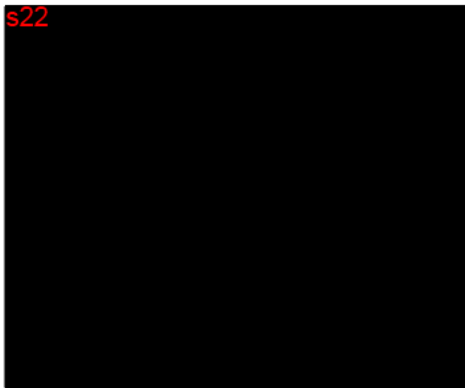
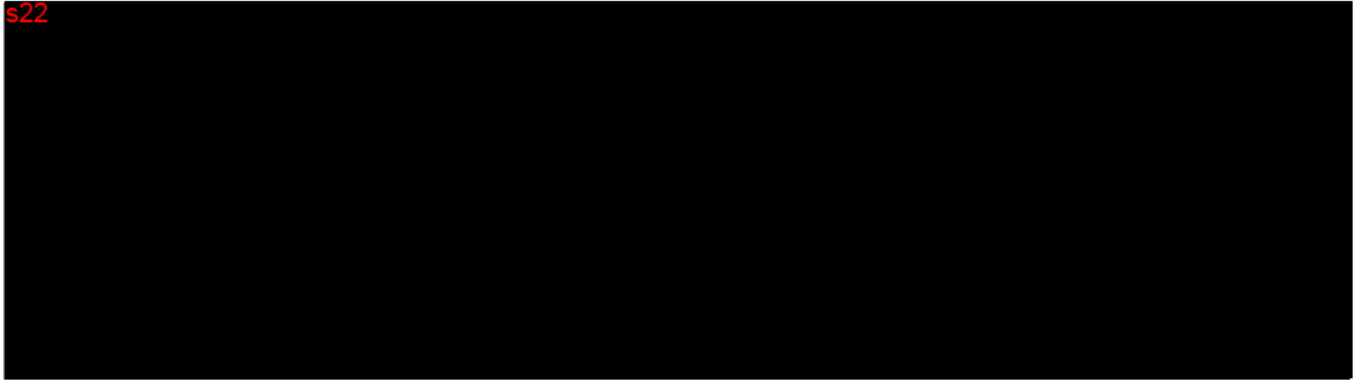
### Gel Image



Filename and data path: C:\Agilent Technologies\Data\2021 10 26\13-56-20\2021 10 26 13H 56M.raw

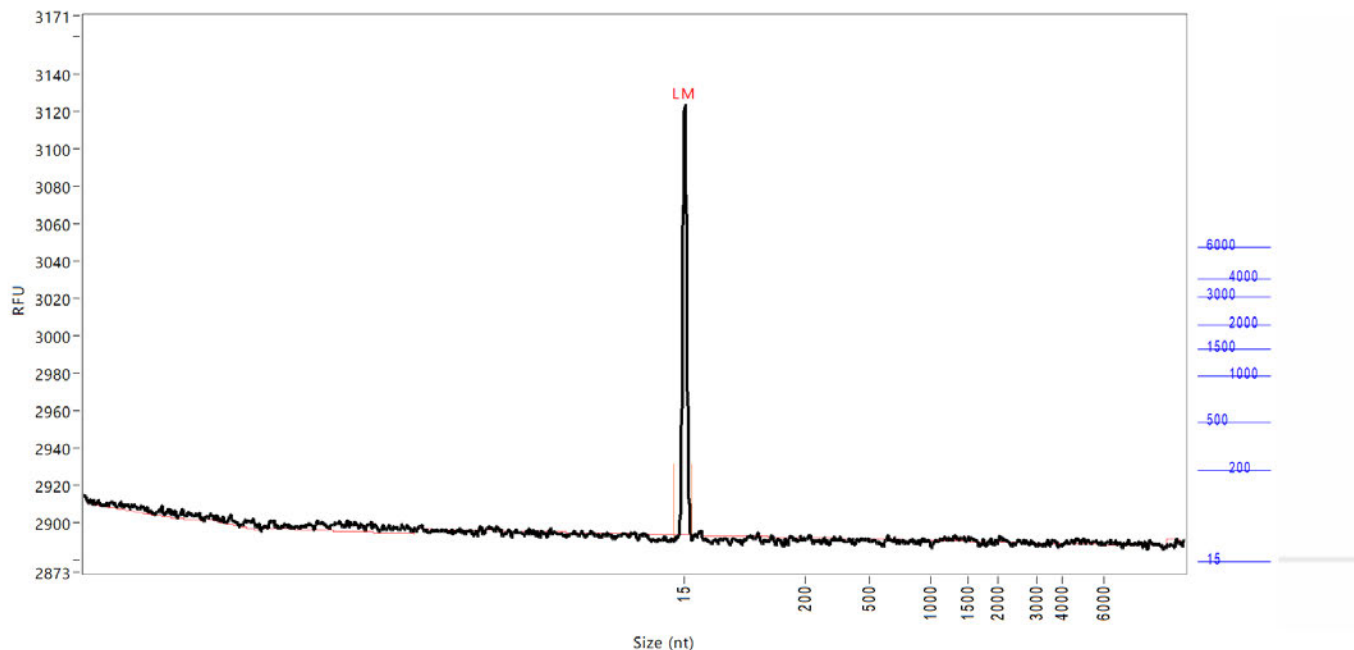


Filename and data path: C:\Agilent Technologies\Data\2021 10 26\13-56-20\2021 10 26 13H 56M.raw





**Sample:** Blank  
**Well location:** A1  
**Created:** Tuesday, October 26, 2021 2:21:46 PM

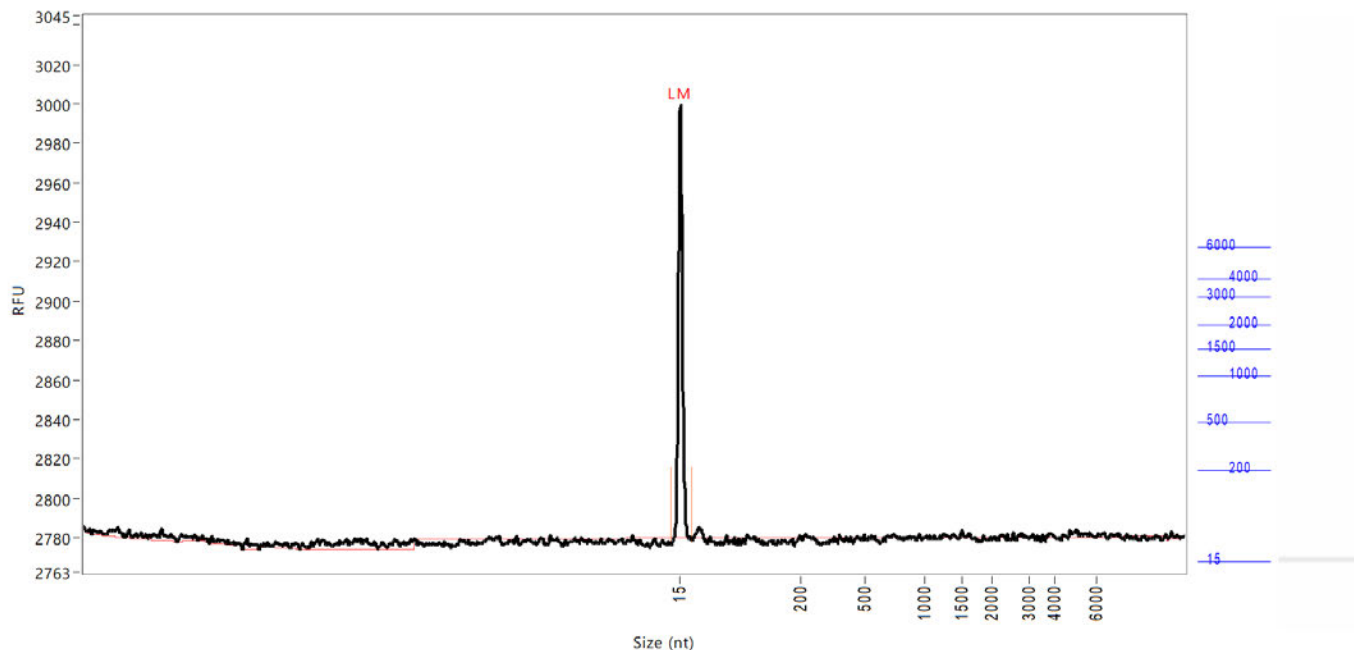


Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.6860	0	26	228
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	0.0282	ng/uL		
	28s/18s:	0.0			
	RQN	1.0			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size (nt)	%CV
	100 nt to 3600 nt	0.0127 ng/ul	45.1 %Total	0.0242 nmole/L	1641 Avg. Size (nt)	21.27 %CV
	3600 nt to 5300 nt	0.0101 ng/ul	35.8 %Total	0.0065 nmole/L	4855 Avg. Size (nt)	3.30 %CV
	5300 nt to 9000 nt	0.0047 ng/ul	16.7 %Total	0.0019 nmole/L	7809 Avg. Size (nt)	10.12 %CV
	9000 nt to 15000 nt	0.0000 ng/ul	0.0 %Total	NaN nmole/L	NaN Avg. Size (nt)	NaN %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Blank  
**Well location:** A2  
**Created:** Tuesday, October 26, 2021 2:21:46 PM

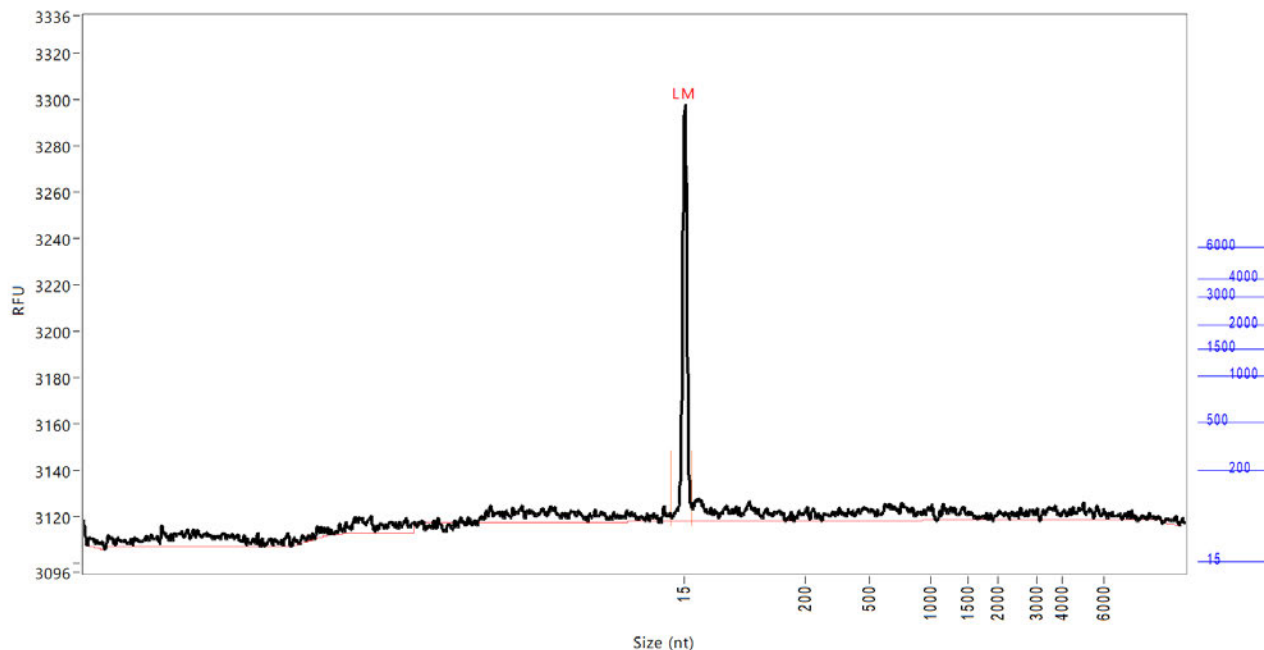


Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.6860	1	34	218
TIC:		0.0000	ng/uL		
TIM:		0.0000	nmole/L		
Total concentration:		0.1578	ng/uL		
28s/18s:		0.0			
RQN		1.0			

Smear Analysis	Size Range (nt)	Concentration (ng/uL)	%Total	Concentration (nmole/L)	Avg. Size (nt)	%CV
	100 nt to 3600 nt	0.0144 ng/uL	9.1 %Total	0.0277 nmole/L	1620 Avg. Size (nt)	22.25 %CV
	3600 nt to 5300 nt	0.0259 ng/uL	16.4 %Total	0.0163 nmole/L	4972 Avg. Size (nt)	2.96 %CV
	5300 nt to 9000 nt	0.0004 ng/uL	0.2 %Total	0.0002 nmole/L	5725 Avg. Size (nt)	0.19 %CV
	9000 nt to 15000 nt	0.0103 ng/uL	6.5 %Total	0.0033 nmole/L	9708 Avg. Size (nt)	0.21 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Blank  
**Well location:** A3  
**Created:** Tuesday, October 26, 2021 2:21:46 PM



Peak	Size	Concentration	From	To	RFU
	(nt)	(ng/uL)	(nt)	(nt)	
1	15 (LM)	0.6860	0	28	178
	TIC:	0.0000	ng/uL		
	TIM:	0.0000	nmole/L		
	Total concentration:	5.0747	ng/uL		
	28s/18s:	0.0			
	RQN	4.7			

Smear Analysis	Size Range	Concentration	%Total	Concentration	Avg. Size	%CV
	100 nt to 3600 nt	3.2225 ng/uL	63.5 %Total	12.3307 nmole/L	815 Avg. Size (nt)	100.75 %CV
	3600 nt to 5300 nt	0.3388 ng/uL	6.7 %Total	0.2360 nmole/L	4479 Avg. Size (nt)	12.00 %CV
	5300 nt to 9000 nt	0.1699 ng/uL	3.3 %Total	0.0889 nmole/L	5964 Avg. Size (nt)	10.93 %CV
	9000 nt to 15000 nt	0.0179 ng/uL	0.4 %Total	0.0059 nmole/L	9522 Avg. Size (nt)	0.62 %CV

Sample peak width (sec): 6    Sample min peak height: 50    Sample baseline V to V?: N    Sample baseline V to V points: 3  
 Sample filter: Binomial    Number of points for filter: 9    Sample start region (min): 0    Sample end region (min): 60  
 Manual baseline start (min): 18    Manual baseline end (min): 59  
 Marker peak width (sec): 6    Marker min peak height: 100    Marker baseline V to V?: Y    Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU    Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder    Final concentration (ng/uL): 8.0000    Dilution factor: 12.0  
 Minimum RFU for data processing: 2

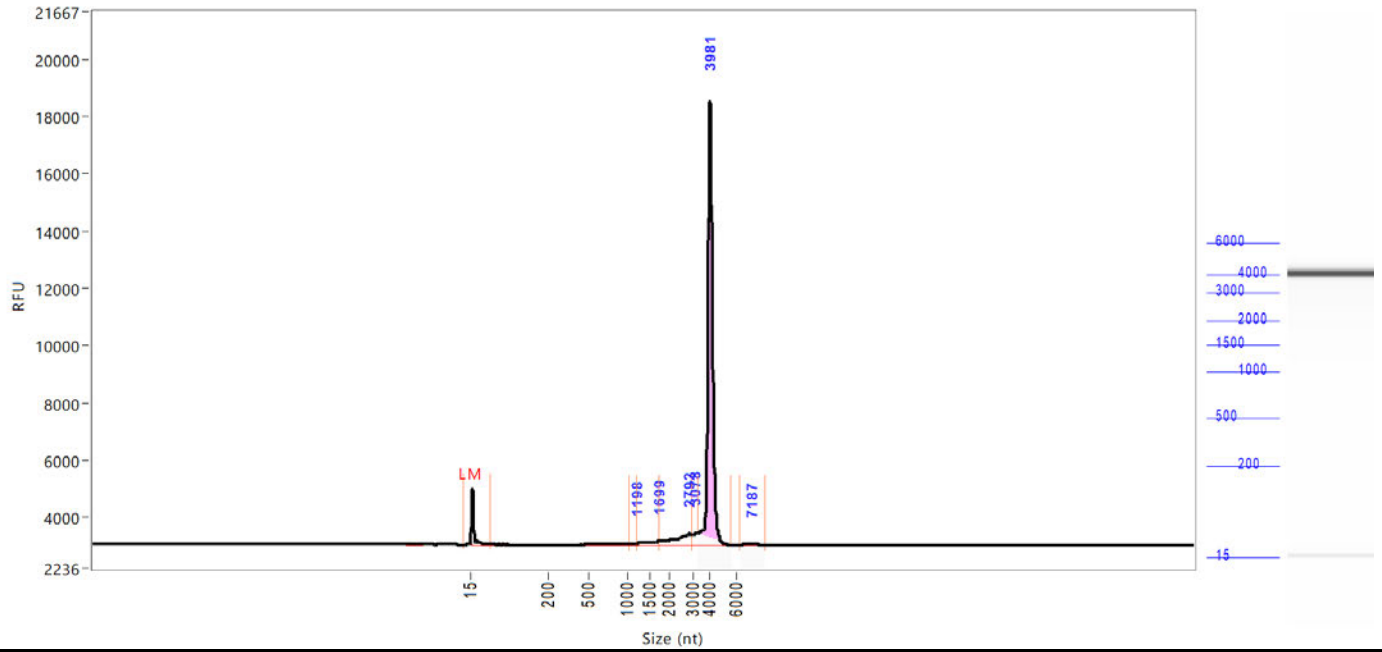






Sample: 000062A-2110003933

S22



S47

s22





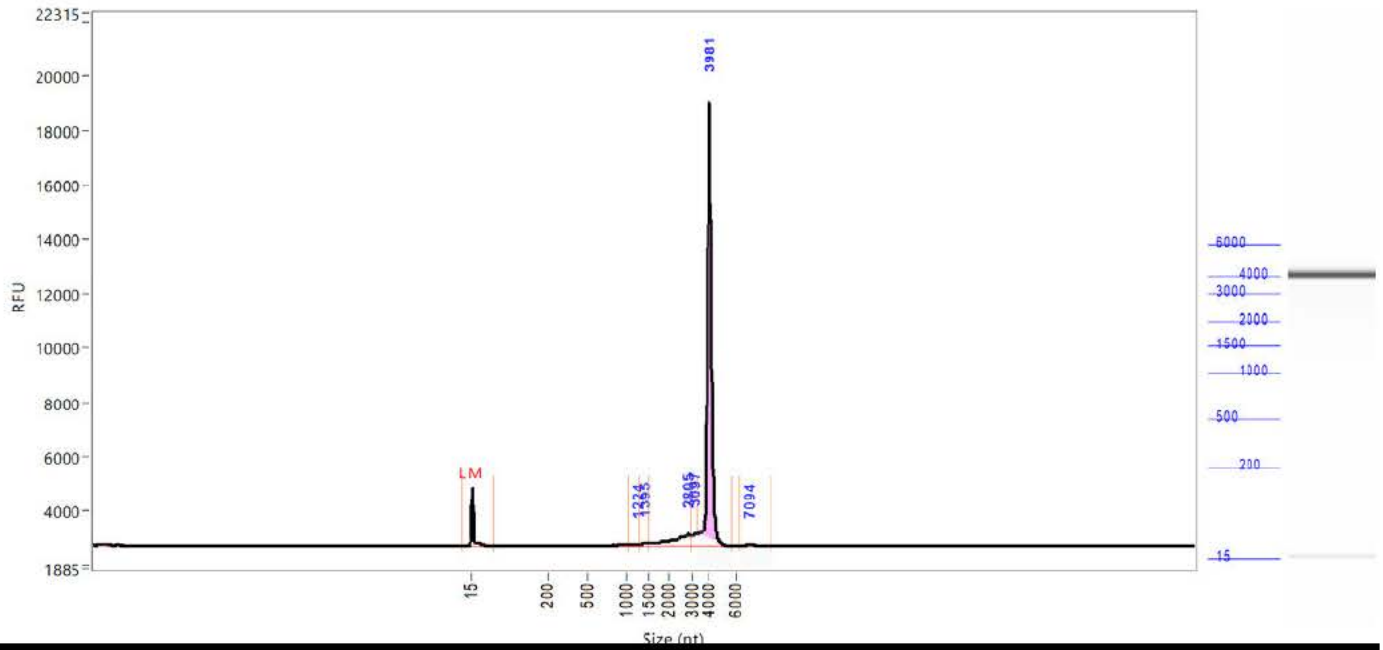






Sample: 000062A-2110003933

s22



s47

s22



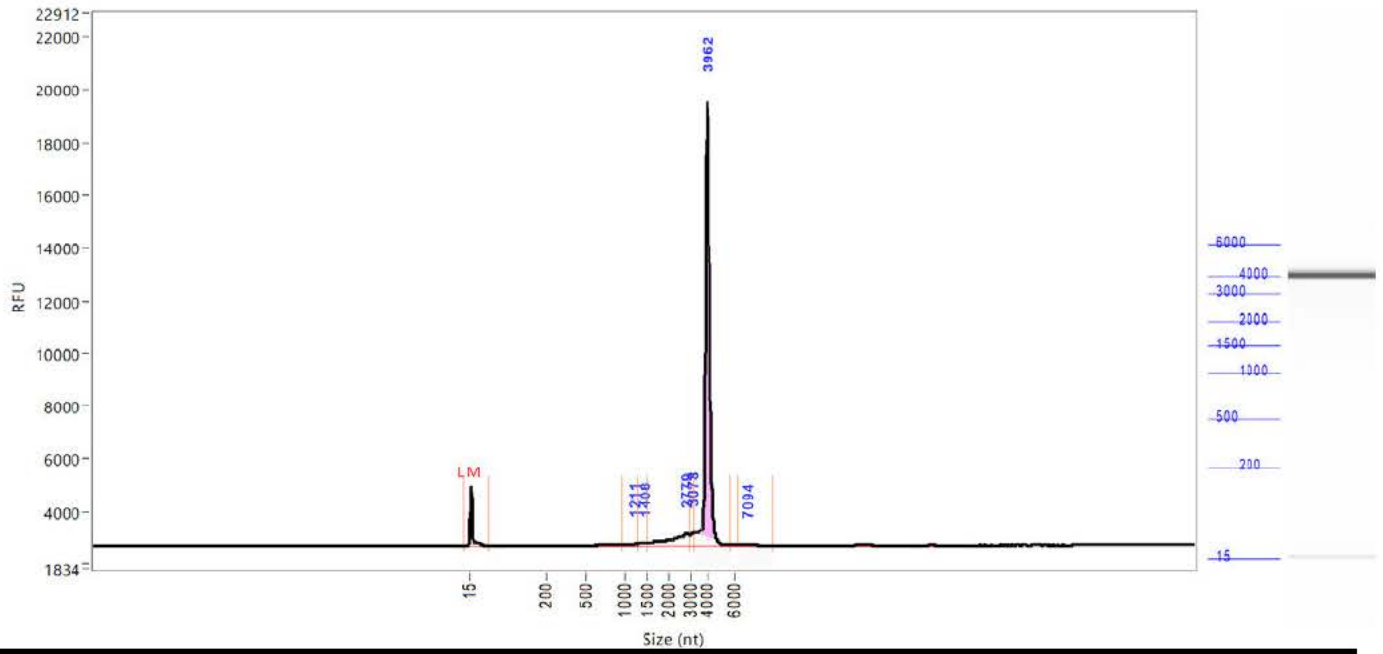








s22

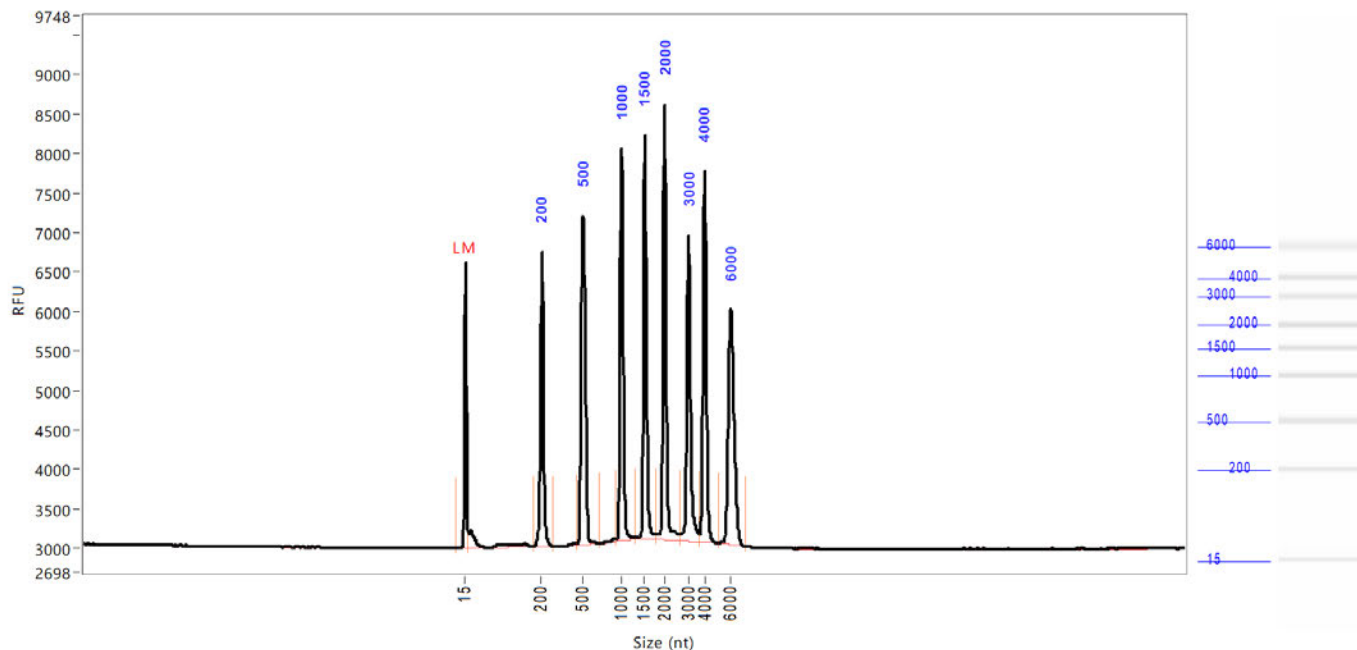


s47

s22



**Sample:** Ladder  
**Well location:** D12  
**Created:** Tuesday, October 26, 2021 2:21:46 PM



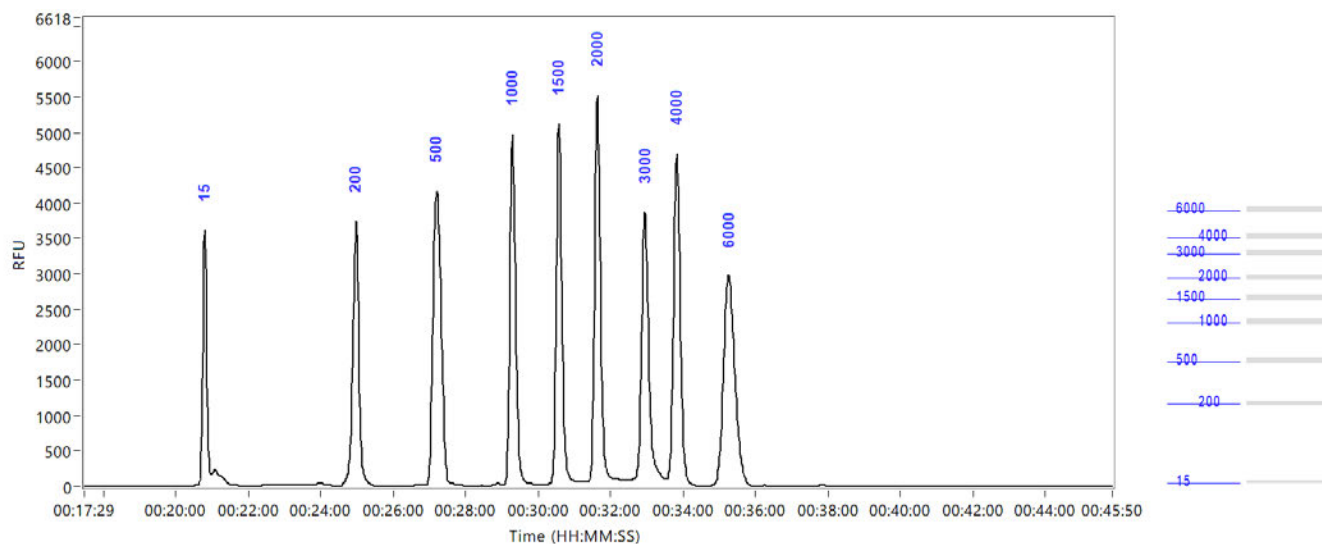
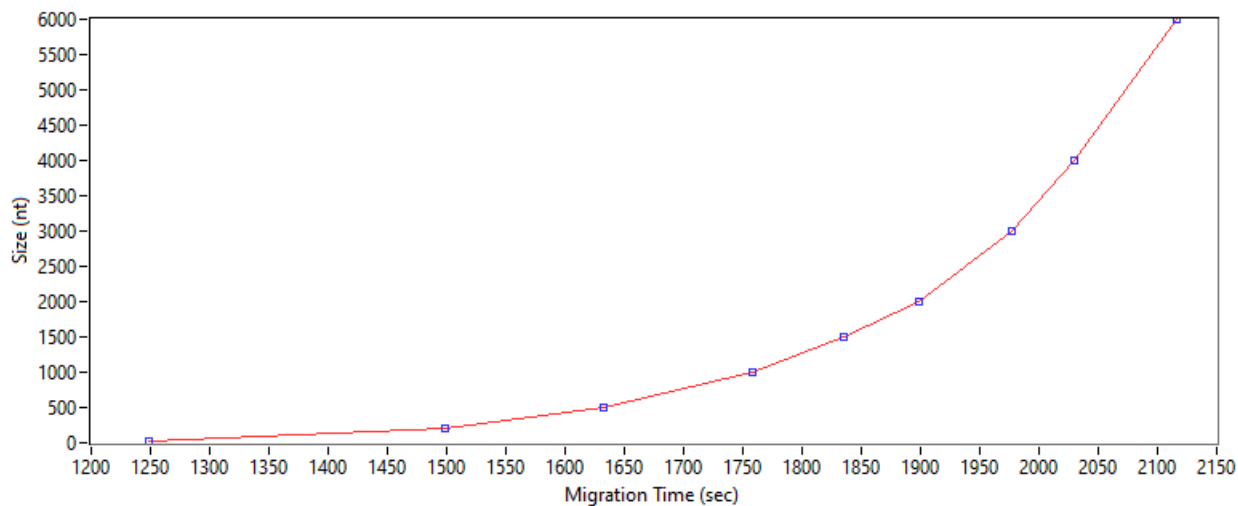
Peak	Size (nt)	Concentration (ng/uL)	From (nt)	To (nt)	RFU
1	15 (LM)	0.6860	0	23	3621
2	200	10.6443	179	280	3738
3	500	14.9622	455	718	4171
4	1000	11.8862	936	1303	4965
5	1500	11.5943	1303	1786	5110
6	2000	13.0994	1786	2650	5513
7	3000	10.7109	2650	3654	3867
8	4000	11.3959	3654	5083	4696
9	6000	11.5542	5083	7094	2981

TIC: 95.8473 ng/uL  
 TIM: 364.5956 nmole/L  
 Total concentration: 96.0000 ng/uL

Sample peak width (sec): 6      Sample min peak height: 200      Sample baseline V to V?: Y      Sample baseline V to V points: 3  
 Sample filter: Binomial      Number of points for filter: 9      Sample start region (min): 0      Sample end region (min): 60  
 Marker peak width (sec): 6      Marker min peak height: 100      Marker baseline V to V?: Y      Marker baseline V to V points: 3  
 Lower marker selection: First peak > 100 RFU      Upper marker selection: Last peak > 100 RFU  
 Ladder size (nt) 15, 200, 500, 1000, 1500, 2000, 3000, 4000, 6000  
 Quantification using: Ladder      Final concentration (ng/uL): 8.0000      Dilution factor: 12.0  
 Minimum RFU for data processing: 2

**Sample:** Ladder  
**Well location:** D12  
**Created:** Tuesday, October 26, 2021 2:21:46 PM  
**Fit type:** Point to point

Calibration curve





C12								
C12								
C12								
D1	D1	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D1	D1	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D1	D1	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN
D1	D1	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN
D2	D2	Blank	9000 nt to 15000 nt	0.0026	0.1	0.0008	9803	0.12
D3	D3	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN
D3	D3	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D4	D4	Blank	5300 nt to 9000 nt	0.002	11.3	0.0007	8345	3.57
D4	D4	Blank	9000 nt to 15000 nt	0.0155	88.7	0.0052	9301	1.09
D5	D5	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN
D5	D5	Blank	9000 nt to 15000 nt	0.0021	8.4	0.0007	9210	0.21
D6	D6	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D6	D6	Blank	3600 nt to 5300 nt	0.013	97.6	0.0088	4579	0.5
D6	D6	Blank	5300 nt to 9000 nt	0.0003	2.4	0.0001	8653	0
D6	D6	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN
D7	D7	Blank	9000 nt to 15000 nt	0.0023	100	0.0008	9214	1.58
D8	D8	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN
D8	D8	Blank	9000 nt to 15000 nt	0.0009	100	0.0003	9037	0.13
D9	D9	Blank	100 nt to 3600 nt	1.3992	68.4	4.8102	907	92.6
D9	D9	Blank	3600 nt to 5300 nt	0.1753	8.6	0.1225	4465	10.33
D9	D9	Blank	5300 nt to 9000 nt	0.0783	3.8	0.0412	5933	9.06
D9	D9	Blank	9000 nt to 15000 nt	0.0042	0.2	0.0014	9171	1.15
D10	D10	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D10	D10	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D10	D10	Blank	5300 nt to 9000 nt	0.0054	84.9	0.0019	8804	2.41
D10	D10	Blank	9000 nt to 15000 nt	0.001	16.1	0.0004	9088	0.15
D11	D11	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN
D11	D11	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN
D11	D11	Blank	5300 nt to 9000 nt	0.003	100	0.0011	8259	6.53
D11	D11	Blank	9000 nt to 15000 nt	0.0001	4	NaN	NaN	NaN
D12	D12	Ladder						

Written By: [Redacted]  
 Authorised: [Redacted]  
 Revision no.: 1  
 LIMS number: 33364

Date Validated: 28/09/2021  
 Validation Due: 28/09/2022  
 Validation Status: **Validation OVERDUE**  
 Analyst: [Redacted]  
 Assay Date: 27/10/2021

1. Enter data on DATA ENTRY TAB,  
 2. Change limits for each smear in columns Z on this tab.  
 3. If a replicate cannot be used, copy and paste data into MANUAL CALCULATIONS tab. The Pass/Fail limits are automatically set from the ALL TRIPLICATE-CALCULATIONS tab.  
 Assay specific Notes:

SMEAR 1										SUMMARY - % TOTAL				Criteria for Reference Material	Criteria for Drug Product
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV			
1	A1	Blank	100 nt to 3600 nt	0.0127	45.1	0.0242	1641	21.27							
2	B1	Blank	100 nt to 3600 nt	0.7182	5.1	1.6852	1329	63.48	Blank	16.7	24.7	147.6	PASS	PASS	
3	C1	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN							
1	A2	Blank	100 nt to 3600 nt	0.0144	9.1	0.0277	1620	22.25							
2	B2	Blank	100 nt to 3600 nt	9.0886	40.6	38.5842	734	93.21	Blank	31.3	19.3	61.7	FAIL	FAIL	
3	C2	Blank	100 nt to 3600 nt	8.9115	44.1	39.1823	709	89.45							
1	A3	Blank	100 nt to 3600 nt	3.2225	63.5	12.3307	815	100.75							
2	B3	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN	Blank	22.5	35.5	157.7	PASS	PASS	
3	C3	Blank	100 nt to 3600 nt	0.1124	4.1	0.323	1085	46.91							
1	A4	Blank	100 nt to 3600 nt	0.0021	0.7	0.0051	1305	4.92							
2	B4	Blank	100 nt to 3600 nt	0.0042	0.7	0.0081	1611	0.35	Blank	1.2	0.9	72.2	PASS	PASS	
3	C4	Blank	100 nt to 3600 nt	0.0131	2.2	0.0299	1373	9.48							
1	A5	Blank	100 nt to 3600 nt	0.1115	7	0.2917	1192	71.16							
2	B5	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN	Blank	2.3	4.0	173.2	PASS	PASS	
3	C5	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN							
1	A9	000062A-2110003933							000062A-2110003933				PASS	PASS	
2	B9	000062A-2110003933													
3	C9	000062A-2110003933													
1	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
2	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
3	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
1	D1	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN	Blank	0.0	0.0	#DIV/0!	PASS	PASS	
2	D2	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN							
3	D3	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN							
1	D4	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN	Blank	0.0	0.0	#DIV/0!	PASS	PASS	
2	D5	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN							
3	D6	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN							
1	D7	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN	Blank	22.8	39.5	173.2	PASS	PASS	
2	D8	Blank	100 nt to 3600 nt	0	0	NaN	NaN	NaN							
3	D9	Blank	100 nt to 3600 nt	1.3992	68.4	4.8102	907	92.6							

SMEAR 2										SUMMARY - % TOTAL				Criteria for Reference Material	Criteria for Drug Product
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV			
1	A1	Blank	3600 nt to 5300 nt	0.0101	35.8	0.0065	4855	3.3							
2	B1	Blank	3600 nt to 5300 nt	0.2202	1.6	0.1545	4447	11.49	Blank	12.5	20.2	162.2	FAIL	FAIL	
3	C1	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN							
1	A2	Blank	3600 nt to 5300 nt	0.0259	16.4	0.0163	4972	2.96							
2	B2	Blank	3600 nt to 5300 nt	1.3832	6.2	0.9463	4560	11.3	Blank	9.0	6.4	71.2	FAIL	FAIL	
3	C2	Blank	3600 nt to 5300 nt	0.9146	4.5	0.6415	4448	10.47							
1	A3	Blank	3600 nt to 5300 nt	0.3388	6.7	0.236	4479	12							
2	B3	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN	Blank	3.3	3.4	101.5	FAIL	FAIL	
3	C3	Blank	3600 nt to 5300 nt	0.0893	3.2	0.0615	4532	10.08							
1	A4	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN							
2	B4	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN	Blank	1.1	2.0	173.2	FAIL	FAIL	
3	C4	Blank	3600 nt to 5300 nt	0.0201	3.4	0.0126	4981	9.22							
1	A5	Blank	3600 nt to 5300 nt	0.0425	2.7	0.0273	4857	5.76							
2	B5	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN	Blank	0.9	1.6	173.2	FAIL	FAIL	
3	C5	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN							
1	A9	000062A-2110003933							000062A-2110003933	82.3	1.1	1.3	PASS	PASS	
2	B9	000062A-2110003933													
3	C9	000062A-2110003933													
1	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	FAIL	FAIL	
2	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	FAIL	FAIL	
3	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	FAIL	FAIL	
1	D1	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN							

ASSUMING SMEAR	
MAIN PEAK	TOTAL IMPURITIES
12.5	23.8
9.0	34.8
3.3	23.8
1.1	3.5
0.9	3.5
81.2	18.9
81.8	18.3
79.8	19.1
82.3	17.7
86.3	13.4
0.0	0.0
0.0	0.0

2	D2	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN	Blank	0.0	0.0	#DIV/0!	FAIL	FAIL
3	D3	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN						
1	D4	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN						
2	D5	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN	Blank	32.5	56.3	173.2	FAIL	FAIL
3	D6	Blank	3600 nt to 5300 nt	0.013	97.6	0.0088	4579	0.5						
1	D7	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN						
2	D8	Blank	3600 nt to 5300 nt	0	0	NaN	NaN	NaN	Blank	2.9	5.0	173.2	FAIL	FAIL
3	D9	Blank	3600 nt to 5300 nt	0.1753	8.6	0.1225	4465	10.33						

0.0	0.0
32.5	36.9
2.9	90.8

SMEAR 3										SUMMARY				Criteria for Reference Material	Criteria for Drug Product
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV			
1	A1	Blank	5300 nt to 9000 nt	0.0047	16.7	0.0019	7809	10.12							
2	B1	Blank	5300 nt to 9000 nt	0.4335	3.1	0.2148	6295	13.56	Blank	6.6	8.9	134.6	PASS	PASS	
3	C1	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN							
1	A2	Blank	5300 nt to 9000 nt	0.0004	0.2	0.0002	5725	0.19							
2	B2	Blank	5300 nt to 9000 nt	0.6819	3	0.3493	6090	9.45	Blank	1.3	1.5	110.6	PASS	PASS	
3	C2	Blank	5300 nt to 9000 nt	0.1624	0.8	0.086	5889	3.63							
1	A3	Blank	5300 nt to 9000 nt	0.1699	3.3	0.0889	5964	10.93							
2	B3	Blank	5300 nt to 9000 nt	0.0027	0	0.0011	7404	0.15	Blank	1.1	1.9	173.2	PASS	PASS	
3	C3	Blank	5300 nt to 9000 nt	0.0008	0	0.0004	5600	0							
1	A4	Blank	5300 nt to 9000 nt	0.0012	0.4	0.0006	6342	0.17							
2	B4	Blank	5300 nt to 9000 nt	0.0063	1.1	0.0028	7155	6.27	Blank	0.8	0.4	45.1	PASS	PASS	
3	C4	Blank	5300 nt to 9000 nt	0.0053	0.9	0.0023	7188	17.25							
1	A5	Blank	5300 nt to 9000 nt	0.0037	0.2	0.0019	6183	14.05							
2	B5	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN	Blank	0.1	0.1	173.2	PASS	PASS	
3	C5	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN							
1	A9	000062A-2110003933							000062A-2110003933				PASS	PASS	
2	B9	000062A-2110003933											PASS	PASS	
3	C9	000062A-2110003933											PASS	PASS	
1	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
2	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
3	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
1	D1	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN	Blank	0.0	0.0	#DIV/0!	PASS	PASS	
2	D2	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN							
3	D3	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN							
1	D4	Blank	5300 nt to 9000 nt	0.002	11.3	0.0007	8345	3.57	Blank	4.6	6.0	130.4	PASS	PASS	
2	D5	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN							
3	D6	Blank	5300 nt to 9000 nt	0.0003	2.4	0.0001	8653	0							
1	D7	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN							
2	D8	Blank	5300 nt to 9000 nt	0	0	NaN	NaN	NaN	Blank	1.3	2.2	173.2	PASS	PASS	
3	D9	Blank	5300 nt to 9000 nt	0.0783	3.8	0.0412	5933	9.06							

SMEAR 4										SUMMARY				Criteria for Reference Material	Criteria for Drug Product
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV			
1	A1	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN							
2	B1	Blank	9000 nt to 15000 nt	0.0784	0.6	0.025	9770	2.55	Blank	0.4	0.4	87.4	PASS	PASS	
3	C1	Blank	9000 nt to 15000 nt	0.014	0.7	0.0048	9167	1.12							
1	A2	Blank	9000 nt to 15000 nt	0.0103	6.5	0.0033	9708	0.21							
2	B2	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN	Blank	2.2	3.8	173.2	PASS	PASS	
3	C2	Blank	9000 nt to 15000 nt	0.0044	0	0.0015	9232	0.14							
1	A3	Blank	9000 nt to 15000 nt	0.0179	0.4	0.0059	9522	0.62							
2	B3	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN	Blank	0.1	0.2	173.2	PASS	PASS	
3	C3	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN							
1	A4	Blank	9000 nt to 15000 nt	0.01	3.4	0.0033	9303	0.9							
2	B4	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN	Blank	1.5	1.7	115.7	PASS	PASS	
3	C4	Blank	9000 nt to 15000 nt	0.0064	1.1	0.0021	9567	1.28							
1	A5	Blank	9000 nt to 15000 nt	0.0445	2.8	0.015	9235	0.91							
2	B5	Blank	9000 nt to 15000 nt	0.023	0.5	0.0077	9394	0.62	Blank	1.1	1.5	135.8	PASS	PASS	
3	C5	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN							
1	A9	000062A-2110003933							000062A-2110003933				PASS	PASS	
2	B9	000062A-2110003933											PASS	PASS	
3	C9	000062A-2110003933											PASS	PASS	
1	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
2	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
3	0	0	0	0	0	0	0	0	0	0.0	0.0	#DIV/0!	PASS	PASS	
1	D1	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN	Blank	0.0	0.1	173.2	PASS	PASS	
2	D2	Blank	9000 nt to 15000 nt	0.0026	0.1	0.0008	9803	0.12							
3	D3	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN							
1	D4	Blank	9000 nt to 15000 nt	0.0155	88.7	0.0052	9301	1.09							



2	D5	Blank	9000 nt to 15000 nt	0.0021	8.4	0.0007	9210	0.21	Blank	32.4	49.0	151.3	FAIL	FAIL
3	D6	Blank	9000 nt to 15000 nt	0	0	NaN	NaN	NaN						
1	D7	Blank	9000 nt to 15000 nt	0.0023	100	0.0008	9214	1.58						
2	D8	Blank	9000 nt to 15000 nt	0.0009	100	0.0003	9037	0.13	Blank	66.7	57.6	86.3	FAIL	FAIL
3	D9	Blank	9000 nt to 15000 nt	0.0042	0.2	0.0014	9171	1.15						

Reference Material Pass/Fail Parameters	
cut off	
S47	

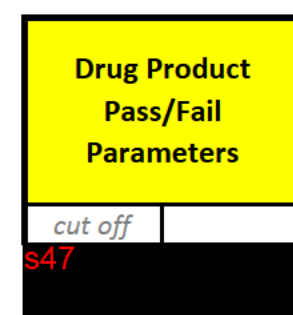
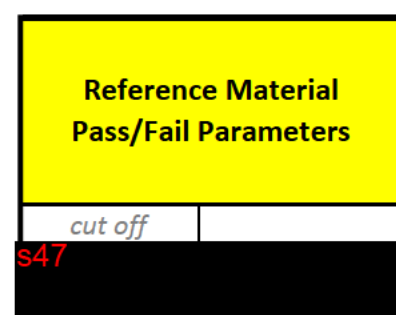
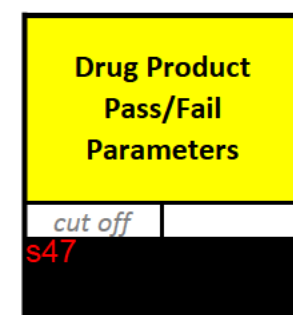
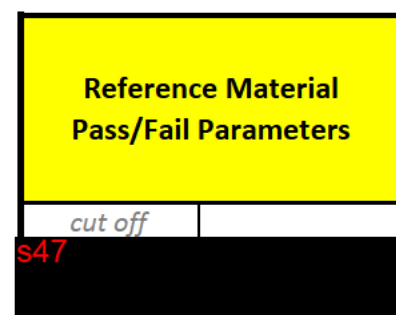
Drug Product Pass/Fail Parameters	
cut off	
S47	

AR 2 IS MAIN PEAK:	
PRE-MAIN PEAK	POST MAIN PEAK
16.7	7.0
31.3	3.5
22.5	1.2
1.2	2.3
2.3	1.2
16.7	2.2
17.4	0.9
16.1	3.0
17.1	0.6
12.8	0.6
0.0	0.0
0.0	0.0

Reference Material Pass/Fail Parameters	
cut off	
S47	

Drug Product Pass/Fail Parameters	
cut off	
S47	

0.0	0.0
0.0	36.9
22.8	68.0



This tab is only to be used if a replace needs to be excluded from the analysis.

Enter raw data directly into this table. All averages/ pass fail will be calculated automatically using the pass/fail parameters entered in the Calculations tab.

SMEAR 1													COMMENTS		
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		Criteria for Reference Material	Criteria for Drug Product
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

SMEAR 2													COMMENTS		
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		Criteria for Reference Material	Criteria for Drug Product
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

SMEAR 3													COMMENTS		
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		Criteria for Reference Material	Criteria for Drug Product
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
3									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	

SMEAR 4													COMMENTS		
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		Criteria for Reference Material	Criteria for Drug Product
1									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	
2									0	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	



Reference Material Pass/Fail Parameters	
cut off	
547	

Drug Product Pass/Fail Parameters	
cut off	
547	

ASSUMING SMEAR 2 IS MAIN PEAK:		
TOTAL IMPURITIES	PRE-MAIN PEAK	POST MAIN PEAK
#DIV/0!	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	#DIV/0!
#DIV/0!	#DIV/0!	#DIV/0!

Reference Material Pass/Fail Parameters	
cut off	
547	

Drug Product Pass/Fail Parameters	
cut off	
547	

Reference Material Pass/Fail Parameters	
cut off	
547	

Drug Product Pass/Fail Parameters	
cut off	
547	

Reference Material Pass/Fail Parameters	
<i>cut off</i>	
547	

Drug Product Pass/Fail Parameters	
<i>cut off</i>	
547	

# DATA ENTRY

A1	A1	Smear 1	20	20	20	20	20
A1	A1	Smear 2	60	60	60	60	60
A1	A1	Smear 3	5	5	5	5	5
A1	A1	Smear 4	5	5	5	5	5
A2	A2	Smear 1	21	21	21	21	21
A2	A2	Smear 2	61	61	61	61	61
A2	A2	Smear 3	6	6	6	6	6
A2	A2	Smear 4	6	6	6	6	6
A3	A3	Smear 1	22	22	22	22	22
A3	A3	Smear 2	62	62	62	62	62
A3	A3	Smear 3	7	7	7	7	7
A3	A3	Smear 4	7	7	7	7	7
A4	A4	Smear 1	23	23	23	23	23
A4	A4	Smear 2	63	63	63	63	63
A4	A4	Smear 3	8	8	8	8	8
A4	A4	Smear 4	8	8	8	8	8
A5	A5	Smear 1	24	24	24	24	24
A5	A5	Smear 2	64	64	64	64	64
A5	A5	Smear 3	9	9	9	9	9
A5	A5	Smear 4	9	9	9	9	9
A6	A6	Smear 1	25	25	25	25	25
A6	A6	Smear 2	65	65	65	65	65
A6	A6	Smear 3	10	10	10	10	10
A6	A6	Smear 4	10	10	10	10	10
A7	A7	Smear 1	26	26	26	26	26
A7	A7	Smear 2	66	66	66	66	66
A7	A7	Smear 3	11	11	11	11	11
A7	A7	Smear 4	11	11	11	11	11
A8	A8	Smear 1	27	27	27	27	27
A8	A8	Smear 2	67	67	67	67	67
A8	A8	Smear 3	12	12	12	12	12
A8	A8	Smear 4	12	12	12	12	12
A9	A9	Smear 1	28	28	28	28	28
A9	A9	Smear 2	68	68	68	68	68
A9	A9	Smear 3	13	13	13	13	13
A9	A9	Smear 4	13	13	13	13	13
A10	A10	Smear 1	29	29	29	29	29
A10	A10	Smear 2	69	69	69	69	69
A10	A10	Smear 3	14	14	14	14	14
A10	A10	Smear 4	14	14	14	14	14
A11	A11	Smear 1	30	30	30	30	30
A11	A11	Smear 2	70	70	70	70	70
A11	A11	Smear 3	15	15	15	15	15
A11	A11	Smear 4	15	15	15	15	15
A12	A12	Smear 1	31	31	31	31	31
A12	A12	Smear 2	71	71	71	71	71
A12	A12	Smear 3	16	16	16	16	16
A12	A12	Smear 4	16	16	16	16	16
B1	B1	Smear 1	20	20	20	20	20
B1	B1	Smear 2	60	60	60	60	60
B1	B1	Smear 3	5	5	5	5	5
B1	B1	Smear 4	5	5	5	5	5
B2	B2	Smear 1	21	21	21	21	21
B2	B2	Smear 2	61	61	61	61	61
B2	B2	Smear 3	6	6	6	6	6
B2	B2	Smear 4	6	6	6	6	6
B3	B3	Smear 1	22	22	22	22	22
B3	B3	Smear 2	62	62	62	62	62
B3	B3	Smear 3	7	7	7	7	7
B3	B3	Smear 4	7	7	7	7	7
B4	B4	Smear 1	23	23	23	23	23
B4	B4	Smear 2	63	63	63	63	63
B4	B4	Smear 3	8	8	8	8	8
B4	B4	Smear 4	8	8	8	8	8
B5	B5	Smear 1	24	24	24	24	24
B5	B5	Smear 2	64	64	64	64	64
B5	B5	Smear 3	9	9	9	9	9
B5	B5	Smear 4	9	9	9	9	9
B6	B6	Smear 1	25	25	25	25	25
B6	B6	Smear 2	65	65	65	65	65
B6	B6	Smear 3	10	10	10	10	10
B6	B6	Smear 4	10	10	10	10	10
B7	B7	Smear 1	26	26	26	26	26
B7	B7	Smear 2	66	66	66	66	66
B7	B7	Smear 3	11	11	11	11	11
B7	B7	Smear 4	11	11	11	11	11
B8	B8	Smear 1	27	27	27	27	27
B8	B8	Smear 2	67	67	67	67	67
B8	B8	Smear 3	12	12	12	12	12
B8	B8	Smear 4	12	12	12	12	12
B9	B9	Smear 1	28	28	28	28	28
B9	B9	Smear 2	68	68	68	68	68
B9	B9	Smear 3	13	13	13	13	13
B9	B9	Smear 4	13	13	13	13	13
B10	B10	Smear 1	29	29	29	29	29
B10	B10	Smear 2	69	69	69	69	69
B10	B10	Smear 3	14	14	14	14	14
B10	B10	Smear 4	14	14	14	14	14
B11	B11	Smear 1	30	30	30	30	30
B11	B11	Smear 2	70	70	70	70	70
B11	B11	Smear 3	15	15	15	15	15
B11	B11	Smear 4	15	15	15	15	15
B12	B12	Smear 1	31	31	31	31	31
B12	B12	Smear 2	71	71	71	71	71
B12	B12	Smear 3	16	16	16	16	16
B12	B12	Smear 4	16	16	16	16	16

# RESULTS

- check that all calculated data highlighted in YELLOW is correct to validate the spreadsheet.  
- check the cut offs (highlighted in orange), then check if the pass/fail results are correct.

SMEAR 1									SUMMARY - % TOTAL				Criteria for Reference Material	Criteria for Drug Product
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
1	A1	A1	Smear 1	20	20	20	20	20	A1	20.0	0.0	0.0	PASS	PASS
2	B1	B1	Smear 1	20	20	20	20	20						
3	C1	C1	Smear 1	20	20	20	20	20						
1	A2	A2	Smear 1	21	21	21	21	21	A2	21.0	0.0	0.0	PASS	PASS
2	B2	B2	Smear 1	21	21	21	21	21						
3	C2	C2	Smear 1	21	21	21	21	21						
1	A3	A3	Smear 1	22	22	22	22	22	A3	22.0	0.0	0.0	PASS	PASS
2	B3	B3	Smear 1	22	22	22	22	22						
3	C3	C3	Smear 1	22	22	22	22	22						
1	A4	A4	Smear 1	23	23	23	23	23	A4	23.0	0.0	0.0	PASS	PASS
2	B4	B4	Smear 1	23	23	23	23	23						
3	C4	C4	Smear 1	23	23	23	23	23						
1	A5	A5	Smear 1	24	24	24	24	24	A5	24.0	0.0	0.0	PASS	PASS
2	B5	B5	Smear 1	24	24	24	24	24						
3	C5	C5	Smear 1	24	24	24	24	24						
1	A6	A6	Smear 1	25	25	25	25	25	A6	25.0	0.0	0.0	PASS	PASS
2	B6	B6	Smear 1	25	25	25	25	25						
3	C6	C6	Smear 1	25	25	25	25	25						
1	A7	A7	Smear 1	26	26	26	26	26	A7	26.0	0.0	0.0	PASS	PASS
2	B7	B7	Smear 1	26	26	26	26	26						
3	C7	C7	Smear 1	26	26	26	26	26						
1	A8	A8	Smear 1	27	27	27	27	27	A8	27.0	0.0	0.0	PASS	PASS
2	B8	B8	Smear 1	27	27	27	27	27						
3	C8	C8	Smear 1	27	27	27	27	27						
1	A9	A9	Smear 1	28	28	28	28	28	A9	28.0	0.0	0.0	FAIL	FAIL
2	B9	B9	Smear 1	28	28	28	28	28						
3	C9	C9	Smear 1	28	28	28	28	28						
1	A10	A10	Smear 1	29	29	29	29	29	A10	29.0	0.0	0.0	FAIL	FAIL
2	B10	B10	Smear 1	29	29	29	29	29						
3	C10	C10	Smear 1	29	29	29	29	29						
1	A11	A11	Smear 1	30	30	30	30	30	A11	30.0	0.0	0.0	FAIL	FAIL
2	B11	B11	Smear 1	30	30	30	30	30						
3	C11	C11	Smear 1	30	30	30	30	30						
1	A12	A12	Smear 1	31	31	31	31	31	A12	31.0	0.0	0.0	FAIL	FAIL
2	B12	B12	Smear 1	31	31	31	31	31						
3	C12	C12	Smear 1	31	31	31	31	31						
1	D1	D1	Smear 1	20	20	20	20	20	D1	21.0	1.0	4.8	PASS	PASS
2	D2	D2	Smear 1	21	21	21	21	21						
3	D3	D3	Smear 1	22	22	22	22	22						
1	D4	D4	Smear 1	23	23	23	23	23	D4	24.0	1.0	4.2	PASS	PASS
2	D5	D5	Smear 1	24	24	24	24	24						
3	D6	D6	Smear 1	25	25	25	25	25						
1	D7	D7	Smear 1	26	26	26	26	26	D7	27.0	1.0	3.7	PASS	PASS
2	D8	D8	Smear 1	27	27	27	27	27						
3	D9	D9	Smear 1	28	28	28	28	28						

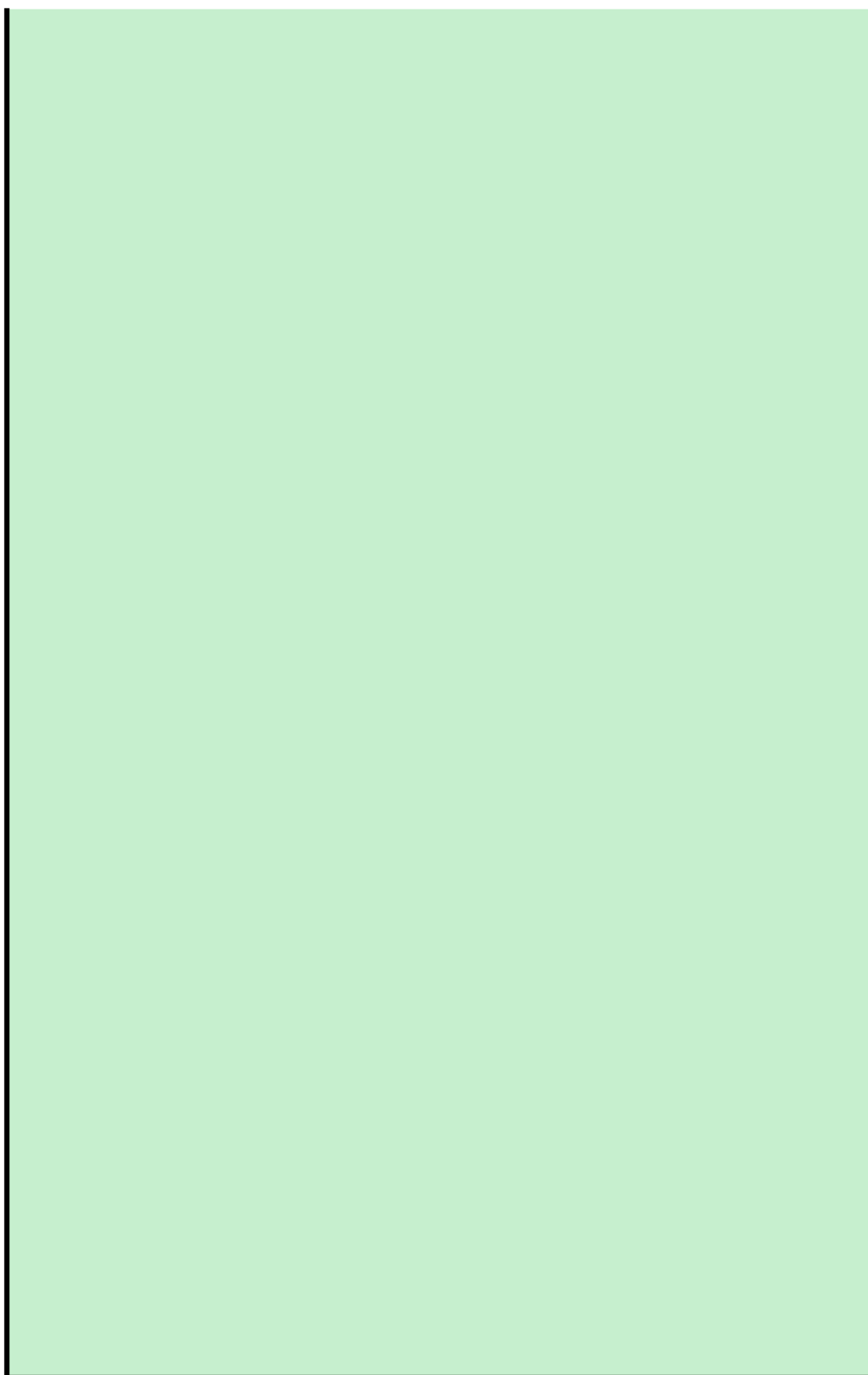
SMEAR 2									SUMMARY - % TOTAL				Criteria for Reference Material	Criteria for Drug Product
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV		
1	A1	A1	Smear 2	60	60	60	60	60	A1	60.0	0.0	0.0	FAIL	FAIL
2	B1	B1	Smear 2	60	60	60	60	60						
3	C1	C1	Smear 2	60	60	60	60	60						
1	A2	A2	Smear 2	61	61	61	61	61	A2	61.0	0.0	0.0	FAIL	FAIL
2	B2	B2	Smear 2	61	61	61	61	61						
3	C2	C2	Smear 2	61	61	61	61	61						
1	A3	A3	Smear 2	62	62	62	62	62	A3	62.0	0.0	0.0	FAIL	FAIL
2	B3	B3	Smear 2	62	62	62	62	62						
3	C3	C3	Smear 2	62	62	62	62	62						
1	A4	A4	Smear 2	63	63	63	63	63	A4	63.0	0.0	0.0	FAIL	FAIL
2	B4	B4	Smear 2	63	63	63	63	63						
3	C4	C4	Smear 2	63	63	63	63	63						
1	A5	A5	Smear 2	64	64	64	64	64	A5	64.0	0.0	0.0	FAIL	FAIL
2	B5	B5	Smear 2	64	64	64	64	64						
3	C5	C5	Smear 2	64	64	64	64	64						
1	A6	A6	Smear 2	65	65	65	65							



B12	B12	Smear 4	16	16	16	16	16	16		
C1	C1	Smear 1	20	20	20	20	20	20		
C1	C1	Smear 2	60	60	60	60	60	60		
C1	C1	Smear 3	5	5	5	5	5	5		
C1	C1	Smear 4	5	5	5	5	5	5		
C2	C2	Smear 1	21	21	21	21	21	21		
C2	C2	Smear 2	61	61	61	61	61	61		
C2	C2	Smear 3	6	6	6	6	6	6		
C2	C2	Smear 4	6	6	6	6	6	6		
C3	C3	Smear 1	22	22	22	22	22	22		
C3	C3	Smear 2	62	62	62	62	62	62		
C3	C3	Smear 3	7	7	7	7	7	7		
C3	C3	Smear 4	7	7	7	7	7	7		
C4	C4	Smear 1	23	23	23	23	23	23		
C4	C4	Smear 2	63	63	63	63	63	63		
C4	C4	Smear 3	8	8	8	8	8	8		
C4	C4	Smear 4	8	8	8	8	8	8		
C5	C5	Smear 1	24	24	24	24	24	24		
C5	C5	Smear 2	64	64	64	64	64	64		
C5	C5	Smear 3	9	9	9	9	9	9		
C5	C5	Smear 4	9	9	9	9	9	9		
C6	C6	Smear 1	25	25	25	25	25	25		
C6	C6	Smear 2	65	65	65	65	65	65		
C6	C6	Smear 3	10	10	10	10	10	10		
C6	C6	Smear 4	10	10	10	10	10	10		
C7	C7	Smear 1	26	26	26	26	26	26		
C7	C7	Smear 2	66	66	66	66	66	66		
C7	C7	Smear 3	11	11	11	11	11	11		
C7	C7	Smear 4	11	11	11	11	11	11		
C8	C8	Smear 1	27	27	27	27	27	27		
C8	C8	Smear 2	67	67	67	67	67	67		
C8	C8	Smear 3	12	12	12	12	12	12		
C8	C8	Smear 4	12	12	12	12	12	12		
C9	C9	Smear 1	28	28	28	28	28	28		
C9	C9	Smear 2	68	68	68	68	68	68		
C9	C9	Smear 3	13	13	13	13	13	13		
C9	C9	Smear 4	13	13	13	13	13	13		
C10	C10	Smear 1	29	29	29	29	29	29		
C10	C10	Smear 2	69	69	69	69	69	69		
C10	C10	Smear 3	14	14	14	14	14	14		
C10	C10	Smear 4	14	14	14	14	14	14		
C11	C11	Smear 1	30	30	30	30	30	30		
C11	C11	Smear 2	70	70	70	70	70	70		
C11	C11	Smear 3	15	15	15	15	15	15		
C11	C11	Smear 4	15	15	15	15	15	15		
C12	C12	Smear 1	31	31	31	31	31	31		
C12	C12	Smear 2	71	71	71	71	71	71		
C12	C12	Smear 3	16	16	16	16	16	16		
C12	C12	Smear 4	16	16	16	16	16	16		
D1	D1	Smear 1	20	20	20	20	20	20		
D1	D1	Smear 2	60	60	60	60	60	60		
D1	D1	Smear 3	5	5	5	5	5	5		
D1	D1	Smear 4	5	5	5	5	5	5		
D2	D2	Smear 1	21	21	21	21	21	21		
D2	D2	Smear 2	61	61	61	61	61	61		
D2	D2	Smear 3	6	6	6	6	6	6		
D2	D2	Smear 4	6	6	6	6	6	6		
D3	D3	Smear 1	22	22	22	22	22	22		
D3	D3	Smear 2	62	62	62	62	62	62		
D3	D3	Smear 3	7	7	7	7	7	7		
D3	D3	Smear 4	7	7	7	7	7	7		
D4	D4	Smear 1	23	23	23	23	23	23		
D4	D4	Smear 2	63	63	63	63	63	63		
D4	D4	Smear 3	8	8	8	8	8	8		
D4	D4	Smear 4	8	8	8	8	8	8		
D5	D5	Smear 1	24	24	24	24	24	24		
D5	D5	Smear 2	64	64	64	64	64	64		
D5	D5	Smear 3	9	9	9	9	9	9		
D5	D5	Smear 4	9	9	9	9	9	9		
D6	D6	Smear 1	25	25	25	25	25	25		
D6	D6	Smear 2	65	65	65	65	65	65		
D6	D6	Smear 3	10	10	10	10	10	10		
D6	D6	Smear 4	10	10	10	10	10	10		
D7	D7	Smear 1	26	26	26	26	26	26		
D7	D7	Smear 2	66	66	66	66	66	66		
D7	D7	Smear 3	11	11	11	11	11	11		
D7	D7	Smear 4	11	11	11	11	11	11		
D8	D8	Smear 1	27	27	27	27	27	27		
D8	D8	Smear 2	67	67	67	67	67	67		
D8	D8	Smear 3	12	12	12	12	12	12		
D8	D8	Smear 4	12	12	12	12	12	12		
D9	D9	Smear 1	28	28	28	28	28	28		
D9	D9	Smear 2	68	68	68	68	68	68		
D9	D9	Smear 3	13	13	13	13	13	13		
D9	D9	Smear 4	13	13	13	13	13	13		
D10	D10	Smear 1	29	29	29	29	29	29		
D10	D10	Smear 2	69	69	69	69	69	69		
D10	D10	Smear 3	14	14	14	14	14	14		
D10	D10	Smear 4	14	14	14	14	14	14		
D11	D11	Smear 1	30	30	30	30	30	30		
D11	D11	Smear 2	70	70	70	70	70	70		
D11	D11	Smear 3	15	15	15	15	15	15		
D11	D11	Smear 4	15	15	15	15	15	15		
D12	D12									

3	D9	D9	Smear 2	68	68	68	68	68									
SMEAR 3									SUMMARY				Criteria for Reference Material	Criteria for Drug Product			
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV					
1	A1	A1	Smear 3	5	5	5	5	5	A1	5.0	0.0	0.0	PASS	PASS			
2	B1	B1	Smear 3	5	5	5	5	5									
3	C1	C1	Smear 3	5	5	5	5	5									
1	A2	A2	Smear 3	6	6	6	6	6	A2	6.0	0.0	0.0	PASS	PASS			
2	B2	B2	Smear 3	6	6	6	6	6									
3	C2	C2	Smear 3	6	6	6	6	6									
1	A3	A3	Smear 3	7	7	7	7	7	A3	7.0	0.0	0.0	FAIL	FAIL			
2	B3	B3	Smear 3	7	7	7	7	7									
3	C3	C3	Smear 3	7	7	7	7	7									
1	A4	A4	Smear 3	8	8	8	8	8	A4	8.0	0.0	0.0	FAIL	FAIL			
2	B4	B4	Smear 3	8	8	8	8	8									
3	C4	C4	Smear 3	8	8	8	8	8									
1	A5	A5	Smear 3	9	9	9	9	9	A5	9.0	0.0	0.0	FAIL	FAIL			
2	B5	B5	Smear 3	9	9	9	9	9									
3	C5	C5	Smear 3	9	9	9	9	9									
1	A6	A6	Smear 3	10	10	10	10	10	A6	10.0	0.0	0.0	FAIL	FAIL			
2	B6	B6	Smear 3	10	10	10	10	10									
3	C6	C6	Smear 3	10	10	10	10	10									
1	A7	A7	Smear 3	11	11	11	11	11	A7	11.0	0.0	0.0	FAIL	FAIL			
2	B7	B7	Smear 3	11	11	11	11	11									
3	C7	C7	Smear 3	11	11	11	11	11									
1	A8	A8	Smear 3	12	12	12	12	12	A8	12.0	0.0	0.0	FAIL	FAIL			
2	B8	B8	Smear 3	12	12	12	12	12									
3	C8	C8	Smear 3	12	12	12	12	12									
1	A9	A9	Smear 3	13	13	13	13	13	A9	13.0	0.0	0.0	FAIL	FAIL			
2	B9	B9	Smear 3	13	13	13	13	13									
3	C9	C9	Smear 3	13	13	13	13	13									
1	A10	A10	Smear 3	14	14	14	14	14	A10	14.0	0.0	0.0	FAIL	FAIL			
2	B10	B10	Smear 3	14	14	14	14	14									
3	C10	C10	Smear 3	14	14	14	14	14									
1	A11	A11	Smear 3	15	15	15	15	15	A11	15.0	0.0	0.0	FAIL	FAIL			
2	B11	B11	Smear 3	15	15	15	15	15									
3	C11	C11	Smear 3	15	15	15	15	15									
1	A12	A12	Smear 3	16	16	16	16	16	A12	16.0	0.0	0.0	FAIL	FAIL			
2	B12	B12	Smear 3	16	16	16	16	16									
3	C12	C12	Smear 3	16	16	16	16	16									
1	D1	D1	Smear 3	5	5	5	5	5	D1	6.0	1.0	16.7	PASS	PASS			
2	D2	D2	Smear 3	6	6	6	6	6									
3	D3	D3	Smear 3	7	7	7	7	7									
1	D4	D4	Smear 3	8	8	8	8	8	D4	9.0	1.0	11.1	FAIL	FAIL			
2	D5	D5	Smear 3	9	9	9	9	9									
3	D6	D6	Smear 3	10	10	10	10	10									
1	D7	D7	Smear 3	11	11	11	11	11	D7	12.0	1.0	8.3	FAIL	FAIL			
2	D8	D8	Smear 3	12	12	12	12	12									
3	D9	D9	Smear 3	13	13	13	13	13									

SMEAR 4									SUMMARY				Criteria for Reference Material	Criteria for Drug Product			
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV					
1	A1	A1	Smear 4	5	5	5	5	5	A1	5.0	0.0	0.0	PASS	PASS			
2	B1	B1	Smear 4	5	5	5	5	5									
3	C1	C1	Smear 4	5	5	5	5	5									
1	A2	A2	Smear 4	6	6	6	6	6	A2	6.0	0.0	0.0	PASS	PASS			
2	B2	B2	Smear 4	6	6	6	6	6									
3	C2	C2	Smear 4	6	6	6	6	6									
1	A3	A3	Smear 4	7	7	7	7	7	A3	7.0	0.0	0.0	FAIL	FAIL			
2	B3	B3	Smear 4	7	7	7	7	7									
3	C3	C3	Smear 4	7	7	7	7	7									
1	A4	A4	Smear 4	8	8	8	8	8	A4	8.0	0.0	0.0	FAIL	FAIL			
2	B4	B4	Smear 4	8	8	8	8	8									
3	C4	C4	Smear 4	8	8	8	8	8									
1	A5	A5	Smear 4	9	9	9	9	9	A5	9.0	0.0						



## DATA ENTRY

SMEAR 1									
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	
1	A1	A1	Smear 1	20	20	20	20	20	
2	B1	B1	Smear 1	20	20	20	20	20	
3	C1	C1	Smear 1	20	20	20	20	20	
1	A2	A2	Smear 1	21	21	21	21	21	
2	B2	B2	Smear 1	21	21	21	21	21	
3	C2	C2	Smear 1	21	21	21	21	21	
1	A3	A3	Smear 1	22	22	22	22	22	
2	B3	B3	Smear 1	22	22	22	22	22	
3	C3	C3	Smear 1	22	22	22	22	22	
1	A4	A4	Smear 1	23	23	23	23	23	
2	B4	B4	Smear 1	23	23	23	23	23	
3	C4	C4	Smear 1	23	23	23	23	23	
1	A5	A5	Smear 1	24	24	24	24	24	
2	B5	B5	Smear 1	24	24	24	24	24	
3	C5	C5	Smear 1	24	24	24	24	24	
1	A6	A6	Smear 1	25	25	25	25	25	
2	B6	B6	Smear 1	25	25	25	25	25	
3	C6	C6	Smear 1	25	25	25	25	25	
1	A7	A7	Smear 1	26	26	26	26	26	
2	B7	B7	Smear 1	26	26	26	26	26	
3	C7	C7	Smear 1	26	26	26	26	26	

SMEAR 2									
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	
1	A1	A1	Smear 2	60	60	60	60	60	
2	B1	B1	Smear 2	60	60	60	60	60	
3	C1	C1	Smear 2	60	60	60	60	60	
1	A2	A2	Smear 2	61	61	61	61	61	
2	B2	B2	Smear 2	61	61	61	61	61	
3	C2	C2	Smear 2	61	61	61	61	61	
1	A3	A3	Smear 2	62	62	62	62	62	
2	B3	B3	Smear 2	62	62	62	62	62	
3	C3	C3	Smear 2	62	62	62	62	62	
1	A4	A4	Smear 2	63	63	63	63	63	
2	B4	B4	Smear 2	63	63	63	63	63	
3	C4	C4	Smear 2	63	63	63	63	63	
1	A5	A5	Smear 2	64	64	64	64	64	
2	B5	B5	Smear 2	64	64	64	64	64	
3	C5	C5	Smear 2	64	64	64	64	64	
1	A6	A6	Smear 2	65	65	65	65	65	
2	B6	B6	Smear 2	65	65	65	65	65	
3	C6	C6	Smear 2	65	65	65	65	65	
1	A7	A7	Smear 2	66	66	66	66	66	
2	B7	B7	Smear 2	66	66	66	66	66	
3	C7	C7	Smear 2	66	66	66	66	66	

SMEAR 3									
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	
1	A1	A1	Smear 3	5	5	5	5	5	
2	B1	B1	Smear 3	5	5	5	5	5	
3	C1	C1	Smear 3	5	5	5	5	5	
1	A2	A2	Smear 3	6	6	6	6	6	
2	B2	B2	Smear 3	6	6	6	6	6	
3	C2	C2	Smear 3	6	6	6	6	6	
1	A3	A3	Smear 3	7	7	7	7	7	
2	B3	B3	Smear 3	7	7	7	7	7	
3	C3	C3	Smear 3	7	7	7	7	7	
1	A4	A4	Smear 3	8	8	8	8	8	
2	B4	B4	Smear 3	8	8	8	8	8	
3	C4	C4	Smear 3	8	8	8	8	8	
1	A5	A5	Smear 3	9	9	9	9	9	
2	B5	B5	Smear 3	9	9	9	9	9	
3	C5	C5	Smear 3	9	9	9	9	9	
1	A6	A6	Smear 3	10	10	10	10	10	
2	B6	B6	Smear 3	10	10	10	10	10	
3	C6	C6	Smear 3	10	10	10	10	10	
1	A7	A7	Smear 3	11	11	11	11	11	
2	B7	B7	Smear 3	11	11	11	11	11	
3	C7	C7	Smear 3	11	11	11	11	11	

SMEAR 4									
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	

## RESULTS

- check that all calculated data highlighted in YELLOW is correct to validate the spreadsheet.
- check the cut offs (highlighted in orange), then check if the pass/fail results are correct.

SMEAR 1															
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	Criteria for Reference Material	Criteria for Drug Product	COMMENTS
1	A1	A1	Smear 1	20	20	20	20	20	A1	20.00	0.00	0.00	PASS	PASS	
2	B1	B1	Smear 1	20	20	20	20	20							
3	C1	C1	Smear 1	20	20	20	20	20							
1	A2	A2	Smear 1	21	21	21	21	21	A2	21.00	0.00	0.00	PASS	PASS	
2	B2	B2	Smear 1	21	21	21	21	21							
3	C2	C2	Smear 1	21	21	21	21	21							
1	A3	A3	Smear 1	22	22	22	22	22	A3	22.00	0.00	0.00	PASS	PASS	
2	B3	B3	Smear 1	22	22	22	22	22							
3	C3	C3	Smear 1	22	22	22	22	22							
1	A4	A4	Smear 1	23	23	23	23	23	A4	23.00	0.00	0.00	PASS	PASS	
2	B4	B4	Smear 1	23	23	23	23	23							
3	C4	C4	Smear 1	23	23	23	23	23							
1	A5	A5	Smear 1	24	24	24	24	24	A5	24.00	0.00	0.00	PASS	PASS	
2	B5	B5	Smear 1	24	24	24	24	24							
3	C5	C5	Smear 1	24	24	24	24	24							
1	A6	A6	Smear 1	25	25	25	25	25	A6	25.00	0.00	0.00	PASS	PASS	
2	B6	B6	Smear 1	25	25	25	25	25							
3	C6	C6	Smear 1	25	25	25	25	25							
1	A7	A7	Smear 1	26	26	26	26	26	A7	26.00	0.00	0.00	PASS	PASS	
2	B7	B7	Smear 1	26	26	26	26	26							
3	C7	C7	Smear 1	26	26	26	26	26							

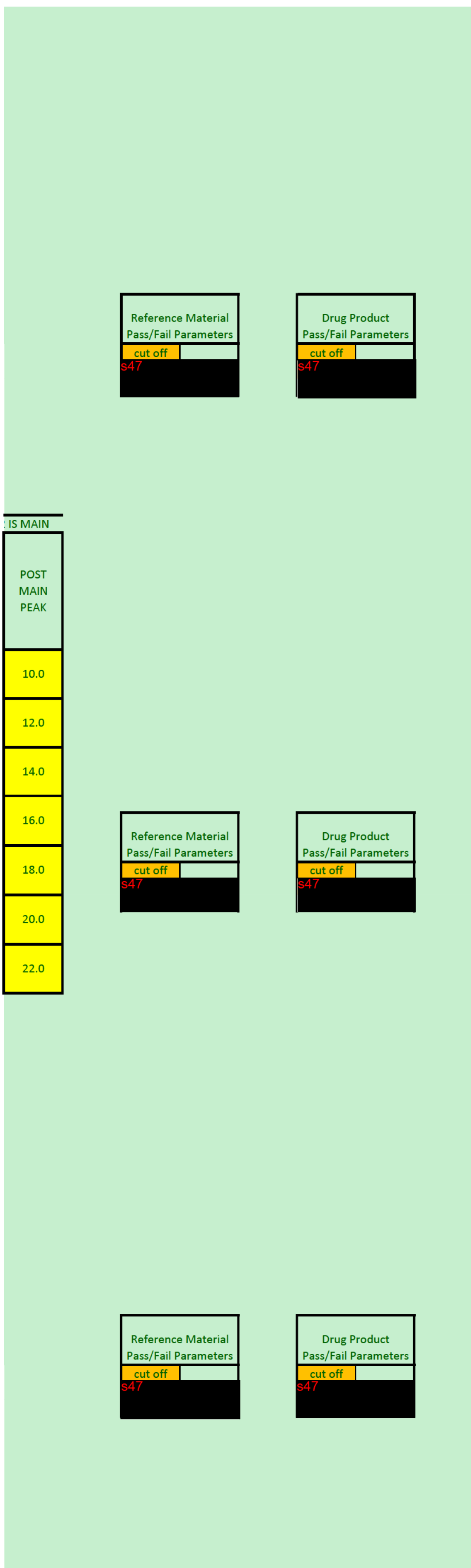
SMEAR 2															
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	Criteria for Reference Material	Criteria for Drug Product	COMMENTS
1	A1	A1	Smear 2	60	60	60	60	60	A1	60.00	0.00	0.00	FAIL	FAIL	
2	B1	B1	Smear 2	60	60	60	60	60							
3	C1	C1	Smear 2	60	60	60	60	60							
1	A2	A2	Smear 2	61	61	61	61	61	A2	61.00	0.00	0.00	FAIL	FAIL	
2	B2	B2	Smear 2	61	61	61	61	61							
3	C2	C2	Smear 2	61	61	61	61	61							
1	A3	A3	Smear 2	62	62	62	62	62	A3	62.00	0.00	0.00	FAIL	FAIL	
2	B3	B3	Smear 2	62	62	62	62	62							
3	C3	C3	Smear 2	62	62	62	62	62							
1	A4	A4	Smear 2	63	63	63	63	63	A4	63.00	0.00	0.00	FAIL	FAIL	
2	B4	B4	Smear 2	63	63	63	63	63							
3	C4	C4	Smear 2	63	63	63	63	63							
1	A5	A5	Smear 2	64	64	64	64	64	A5	64.00	0.00	0.00	FAIL	FAIL	
2	B5	B5	Smear 2	64	64	64	64	64							
3	C5	C5	Smear 2	64	64	64	64	64							
1	A6	A6	Smear 2	65	65	65	65	65	A6	65.00	0.00	0.00	PASS	PASS	
2	B6	B6	Smear 2	65	65	65	65	65							
3	C6	C6	Smear 2	65	65	65	65	65							
1	A7	A7	Smear 2	66	66	66	66	66	A7	66.00	0.00	0.00	PASS	PASS	
2	B7	B7	Smear 2	66	66	66	66	66							
3	C7	C7	Smear 2	66	66	66	66	66							

SMEAR 3															
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	Criteria for Reference Material	Criteria for Drug Product	COMMENTS
1	A1	A1	Smear 3	5	5	5	5	5	A1	5.00	0.00	0.00	PASS	PASS	
2	B1	B1	Smear 3	5	5	5	5	5							
3	C1	C1	Smear 3	5	5	5	5	5							
1	A2	A2	Smear 3	6	6	6	6	6	A2	6.00	0.00	0.00	PASS	PASS	
2	B2	B2	Smear 3	6	6	6	6	6							
3	C2	C2	Smear 3	6	6	6	6	6							
1	A3	A3	Smear 3	7	7	7	7	7	A3	7.00	0.00	0.00	FAIL	FAIL	
2	B3	B3	Smear 3	7	7	7	7	7							
3	C3	C3	Smear 3	7	7	7	7	7							
1	A4	A4	Smear 3	8	8	8	8	8	A4	8.00	0.00	0.00	FAIL	FAIL	
2	B4	B4	Smear 3	8	8	8	8	8							
3	C4	C4	Smear 3	8	8	8	8	8							
1	A5	A5	Smear 3	9	9	9	9	9	A5	9.00	0.00	0.00	FAIL	FAIL	
2	B5	B5	Smear 3	9	9	9	9	9							
3	C5	C5	Smear 3	9	9	9	9	9							
1	A6	A6	Smear 3	10	10	10	10	10	A6	10.00	0.00	0.00	FAIL	FAIL	
2	B6	B6	Smear 3	10	10	10	10	10							
3	C6	C6	Smear 3	10	10	10	10	10							
1	A7	A7	Smear 3	11	11	11	11	11	A7	11.00	0.00	0.00	FAIL	FAIL	
2	B7	B7	Smear 3	11	11	11	11	11							
3	C7	C7	Smear 3	11	11	11	11	11							

SMEAR 4														
REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV						

1	A1	A1	Smear 4	5	5	5	5	5		
2	B1	B1	Smear 4	5	5	5	5	5		
3	C1	C1	Smear 4	5	5	5	5	5		
1	A2	A2	Smear 4	6	6	6	6	6		
2	B2	B2	Smear 4	6	6	6	6	6		
3	C2	C2	Smear 4	6	6	6	6	6		
1	A3	A3	Smear 4	7	7	7	7	7		
2	B3	B3	Smear 4	7	7	7	7	7		
3	C3	C3	Smear 4	7	7	7	7	7		
1	A4	A4	Smear 4	8	8	8	8	8		
2	B4	B4	Smear 4	8	8	8	8	8		
3	C4	C4	Smear 4	8	8	8	8	8		
1	A5	A5	Smear 4	9	9	9	9	9		
2	B5	B5	Smear 4	9	9	9	9	9		
3	C5	C5	Smear 4	9	9	9	9	9		
1	A6	A6	Smear 4	10	10	10	10	10		
2	B6	B6	Smear 4	10	10	10	10	10		
3	C6	C6	Smear 4	10	10	10	10	10		
1	A7	A7	Smear 4	11	11	11	11	11		
2	B7	B7	Smear 4	11	11	11	11	11		
3	C7	C7	Smear 4	11	11	11	11	11		

REPLICATE	Well	Sample ID	Range	ng/uL	% Total	nmole/L	Avg. Size	%CV	Sample ID	Average	stdev	%CV	Criteria for Reference Material	Criteria for Drug Product
1	A1	A1	Smear 4	5	5	5	5	5	A1	5.00	0.00	0.00	PASS	PASS
2	B1	B1	Smear 4	5	5	5	5	5						
3	C1	C1	Smear 4	5	5	5	5	5						
1	A2	A2	Smear 4	6	6	6	6	6	A2	6.00	0.00	0.00	PASS	PASS
2	B2	B2	Smear 4	6	6	6	6	6						
3	C2	C2	Smear 4	6	6	6	6	6						
1	A3	A3	Smear 4	7	7	7	7	7	A3	7.00	0.00	0.00	FAIL	FAIL
2	B3	B3	Smear 4	7	7	7	7	7						
3	C3	C3	Smear 4	7	7	7	7	7						
1	A4	A4	Smear 4	8	8	8	8	8	A4	8.00	0.00	0.00	FAIL	FAIL
2	B4	B4	Smear 4	8	8	8	8	8						
3	C4	C4	Smear 4	8	8	8	8	8						
1	A5	A5	Smear 4	9	9	9	9	9	A5	9.00	0.00	0.00	FAIL	FAIL
2	B5	B5	Smear 4	9	9	9	9	9						
3	C5	C5	Smear 4	9	9	9	9	9						
1	A6	A6	Smear 4	10	10	10	10	10	A6	10.00	0.00	0.00	FAIL	FAIL
2	B6	B6	Smear 4	10	10	10	10	10						
3	C6	C6	Smear 4	10	10	10	10	10						
1	A7	A7	Smear 4	11	11	11	11	11	A7	11.00	0.00	0.00	FAIL	FAIL
2	B7	B7	Smear 4	11	11	11	11	11						
3	C7	C7	Smear 4	11	11	11	11	11						



Reference Material	
Pass/Fail Parameters	
cut off	
s47	

Drug Product	
Pass/Fail Parameters	
cut off	
s47	

RM-2107002766 DH-03180.1

1	3981
2	3962
3	3962
avg	3968
stdev	11
%RSD	0.28



Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

<b>Owner:</b> s22	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b>	<b>Review:</b>
<b>Title:</b>	

### Worksheet for Fragment Analyzer

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-26	<b>Analyst</b>	s22
<b>TRIM link to data files</b>	D21-3263441, D21-3263435	<b>Test Date</b>	26/10/2021
<b>Modifications to SOP</b>	See report <a href="#">D21-3175558</a> - DRAFT - Spikevax (Moderna) - Method Modifications - mRNA purity - Fragment Analyzer - Capillary Gel Electrophoresis - 05Oct2021 - contem		

Pipettes & Equipment	
Name	LIMS#
P20	32891
P100	32792
P200	5649
thermomixer	23660
Enter text.	Enter text.
Enter text.	Enter text.
Enter text.	Enter text.

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	-
Inlet Buffer	DNF-355-0300	0006593943	1/03/2022
Rinse Buffer	DNF-497-0125	0006588740	2/02/2022
Capillary Storage Buffer	GP-440-0100	0006578989	14/12/2022
Capillary conditioning solution	DNF-475-0100	0006598614	22/03/2022
RNA Separation Gel	DNF-265-0500	0006597220	15/04/2022
Intercalating dye	DNF-600-U030	6603014	9/04/2022
Blank	DNF-300-0008	6595951	10/03/2022
RNA Ladder	DNF-382-U020	0006600148	28/03/2022
Diluent Marker	DNF-369-0004	0006602443	7/04/2023
DEPC water	AM9961	2004017	/N/A
20% T-X100 / 30% EtOH solution	In House	MC1SEP21-01	1/02/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Enter text.	Enter text.	Enter text.	Enter a date.



Enter text.	Enter text.	Enter text.	Enter a date.
Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT (Drawer B)	26/10/2021	27/10/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT (Drawer M)	26/10/2021	27/10/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT (Drawer 3)	26/10/2021	9/11/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	26/10/2021	9/11/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	26/10/2021	28/10/2021  48 hours
<b>Empty waste tray and waste bottle</b> Reagents can be scaled up if required – this table provides the minimum for a single run. Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.			

## 96 well Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S11a	S10a	S9a	S8a	S7a	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	S11b	S10b	S9b	S8b	S7b	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	S11c	S10c	S9c	S8c	S7c	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	S12a	S12b	S12c	S13a	S13b	S13c	S14a	S14b	S14c	BF-25	BF-25	L

**S1-14** = Samples in triplicate (a, b or c), note this worksheet only contains enough fields for 6 samples.

**RM** = Reference material

**BF-25** = Blank solution provided in kit,

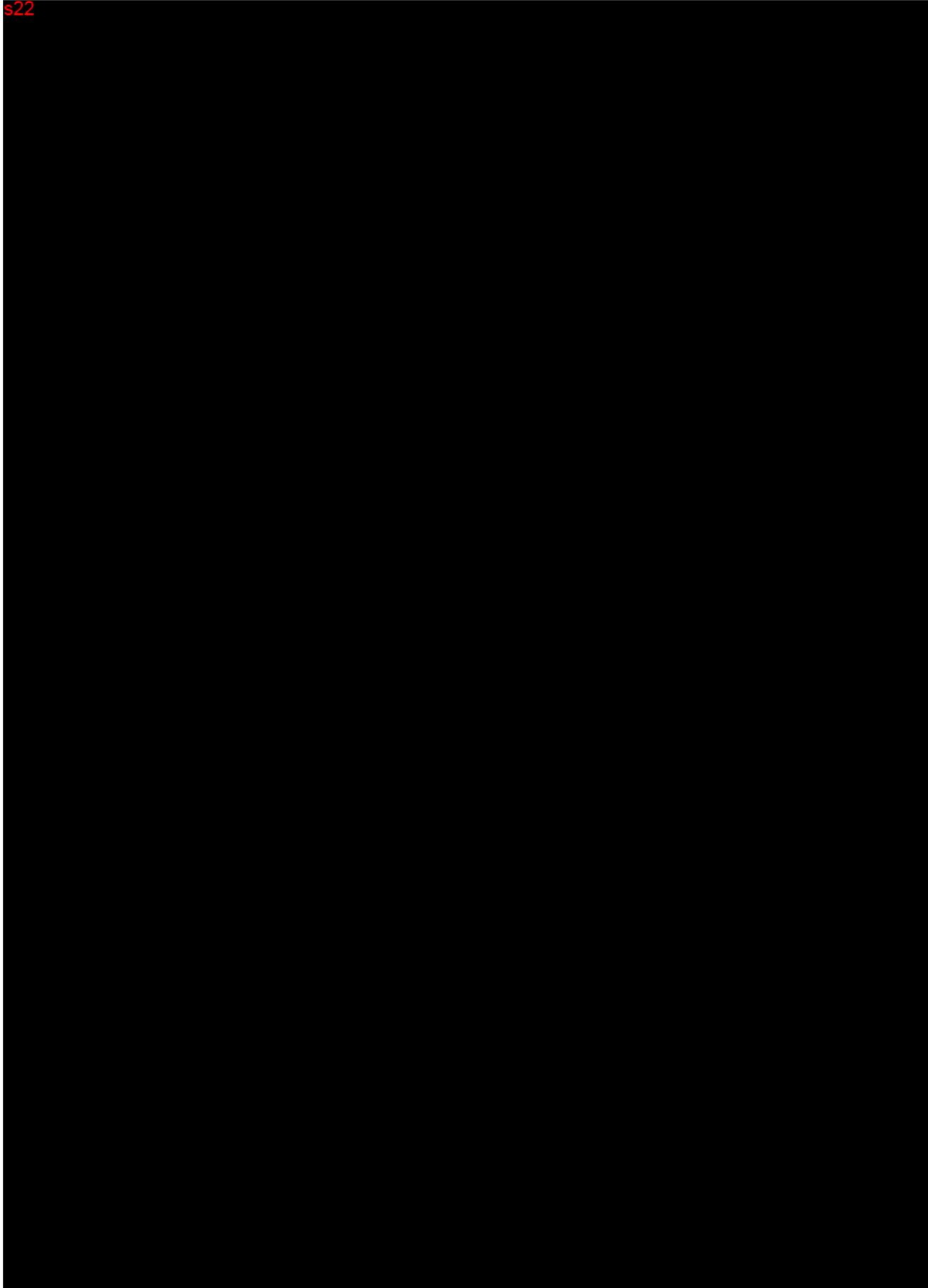
**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test.

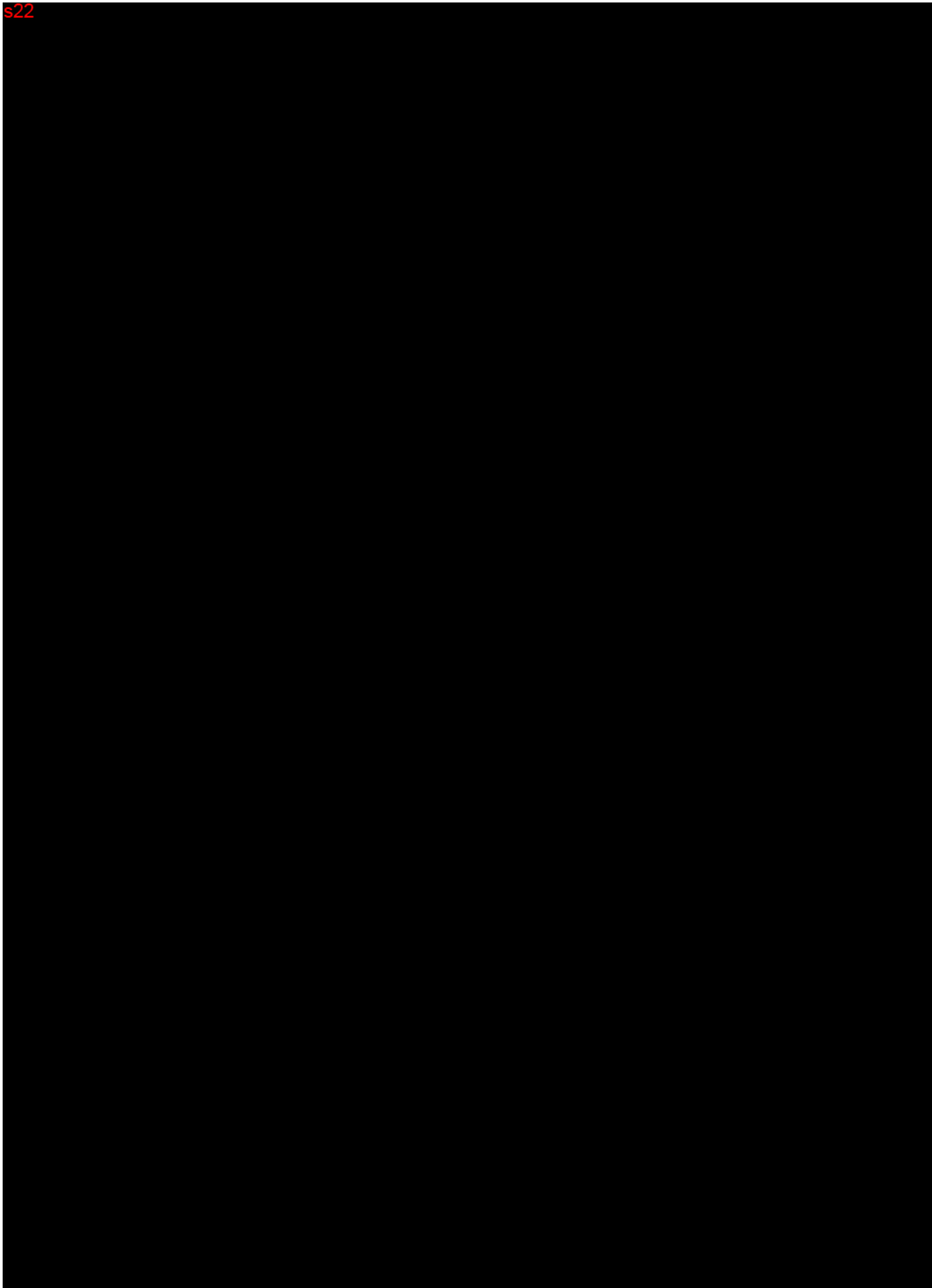
System Suitability Criteria					
Tested	Well	Parameter	Limits	Results	PASS/FAIL
Ladder	D12	Ladder Profile	Must be comparable to Figure 7 D21-3175558	ok	PASS
Ladder	D12	Peaks Present	All peaks must be present	ok	PASS
Ladder	D12	Peak Height	<60,000 RFU	ok	PASS
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Choose an item.	Enter text.	Enter text.	Enter text.	Choose an item.

Assay Acceptance Criteria					
Tested RM (LIMS), blank, ladder?	Well	Parameter	Limits	Results	PASS/FAIL
RM 2107002766 DH-03180.1	A10 B10 C10	Profile	Similar to Figure 5 D21-3175558	OK/ok/ok	PASS
RM 2107002766 DH-03180.1	A10 B10 C10	% RSD of the % Total for the main peak smear range	<5%	1.7	PASS
RM 2107002766 DH-03180.1	A10 B10 C10	% RSD of the main peak fragment size (nt)	<5%	(3981/3962/3962), 0.28	PASS
RM 2107002766 DH-03180.1	A10 B10 C10	Lower Marker	Must be present	OK/ok/ok	PASS
RM 2107002766 DH-03180.1	A10 B10 C10	Peak heights	5000-60,000 RFU for 2/3 replicates	20958/21189/18339	PASS
RM 2107002766 DH-03180.1	A10 B10 C10	Negative Peaks/Baseline shifts	None present in at least 2/3 replicates	None present	PASS
Dilutions / Calculation / Notes					
Reference Material prep - 2107002766 DH-03180.1					
1. Diluted to ~200 ng/uL with DEPC = 13 uL of 3000 ng/uL + 187 uL DPEC					
2. Dilute to 67 ng/uL with DEPC = 20 uL of 200 ng/uL + 40 uL DEPC					
12 uL of 67 ng/uL in well A12 + 132 uL Diluent marker.					

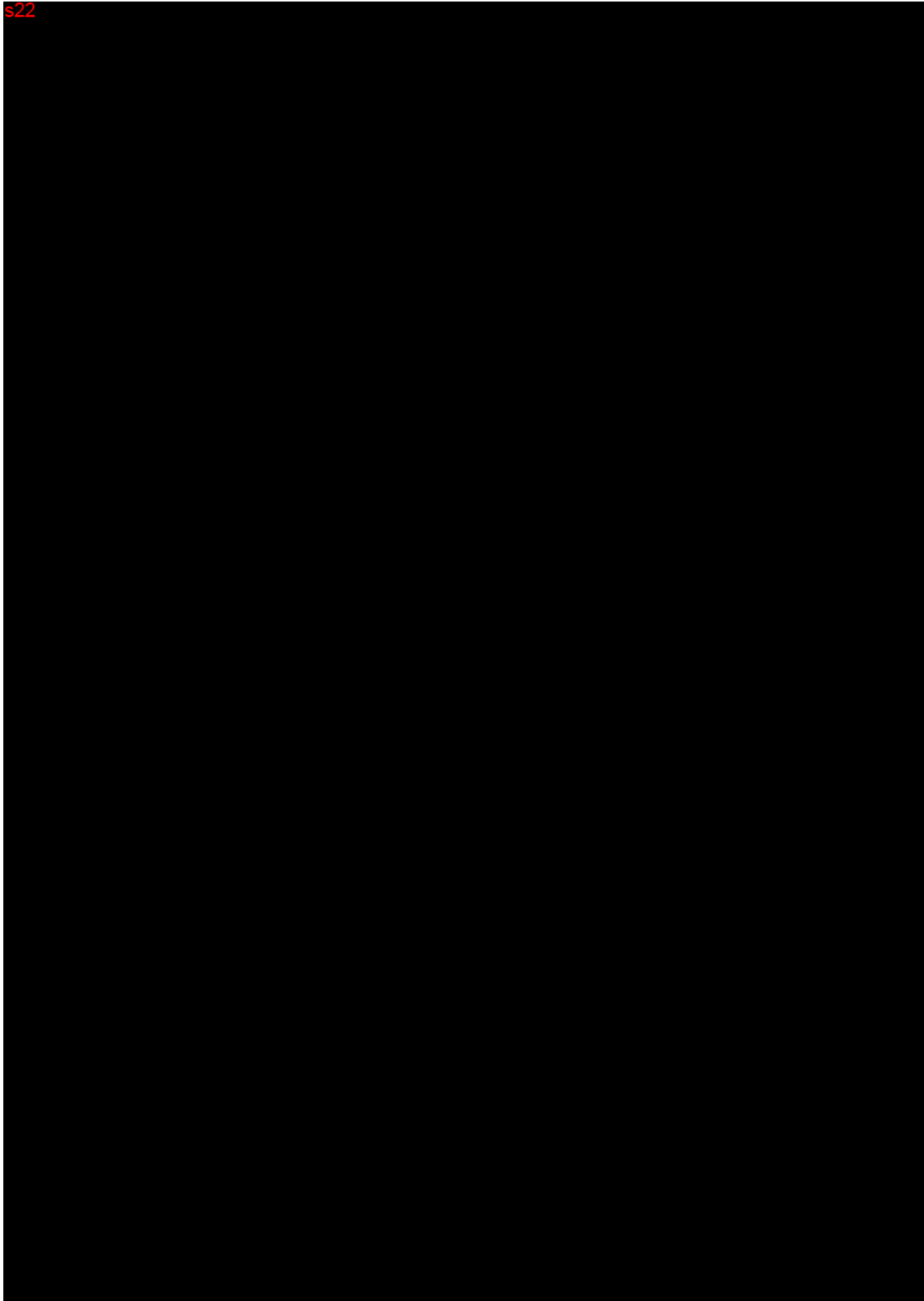
s22



s22



S22

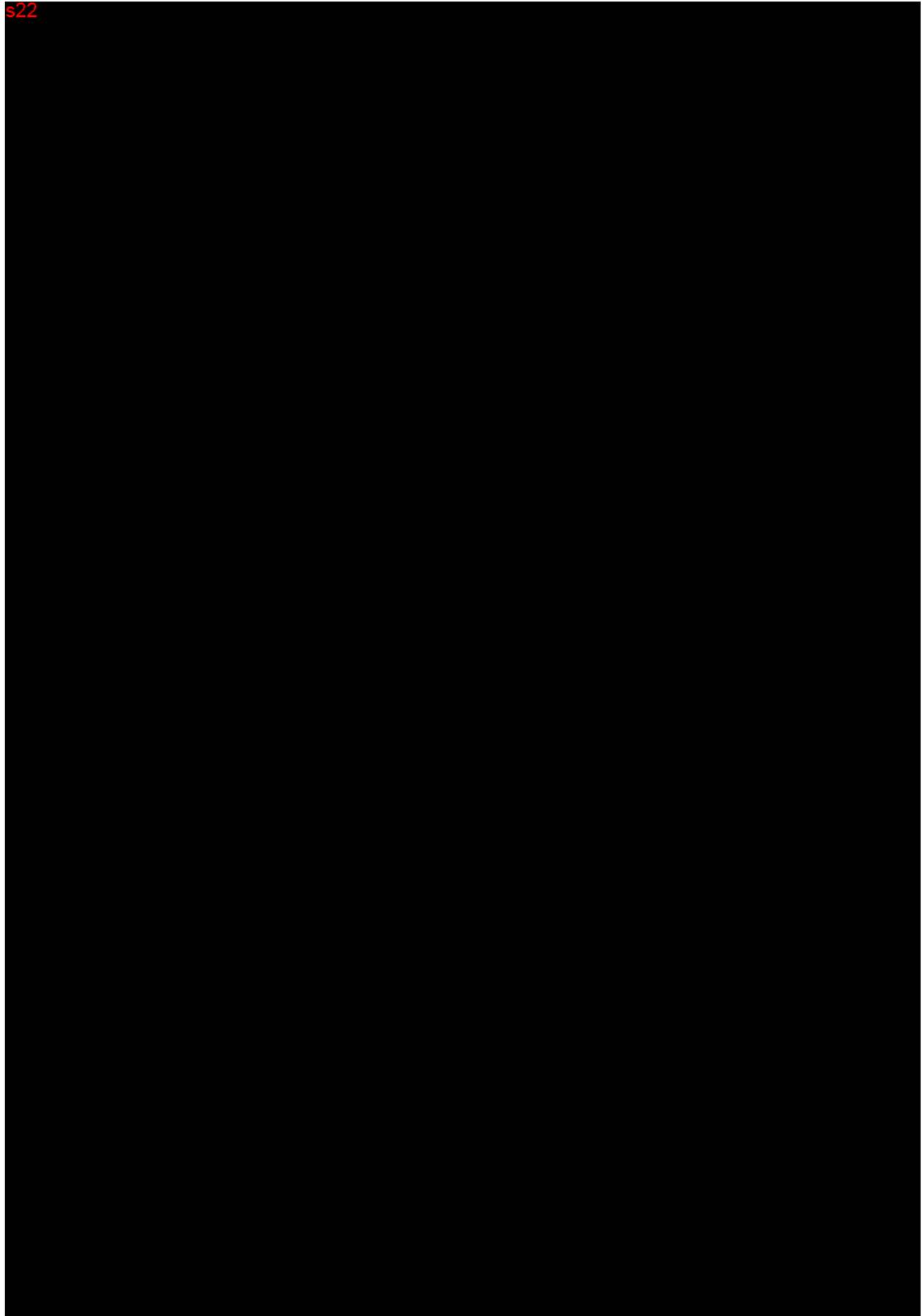


Sample 4					
Plate location (wells)	A9 B9 C9				
LIMS #	2110003933				
BATCH #	000062A				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
% RSD of the % Total for the main peak smear range	≤5%.	1.3			PASS
Peak height	5000-60,000RFU	15521/16327/16838			PASS
Main peak fragment size (nt)	Comparable to RM	3981/3981/3962			PASS
Lower Marker	Must be present	OK/ok/ok			PASS
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Main Peak	s47	s47			PASS
Impurity group 1 – RNA fragments					PASS
Impurity group 2 – Poly A tail variant					PASS
Impurity group 3 – RNA lipid adduct					PASS
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
<b>Choose an item.</b>					
Analysist			[Redacted]		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
20 uL of 200 ng/uL + 40 uL Tx100/30% ethanol = 60 uL of 67 ng/uL					

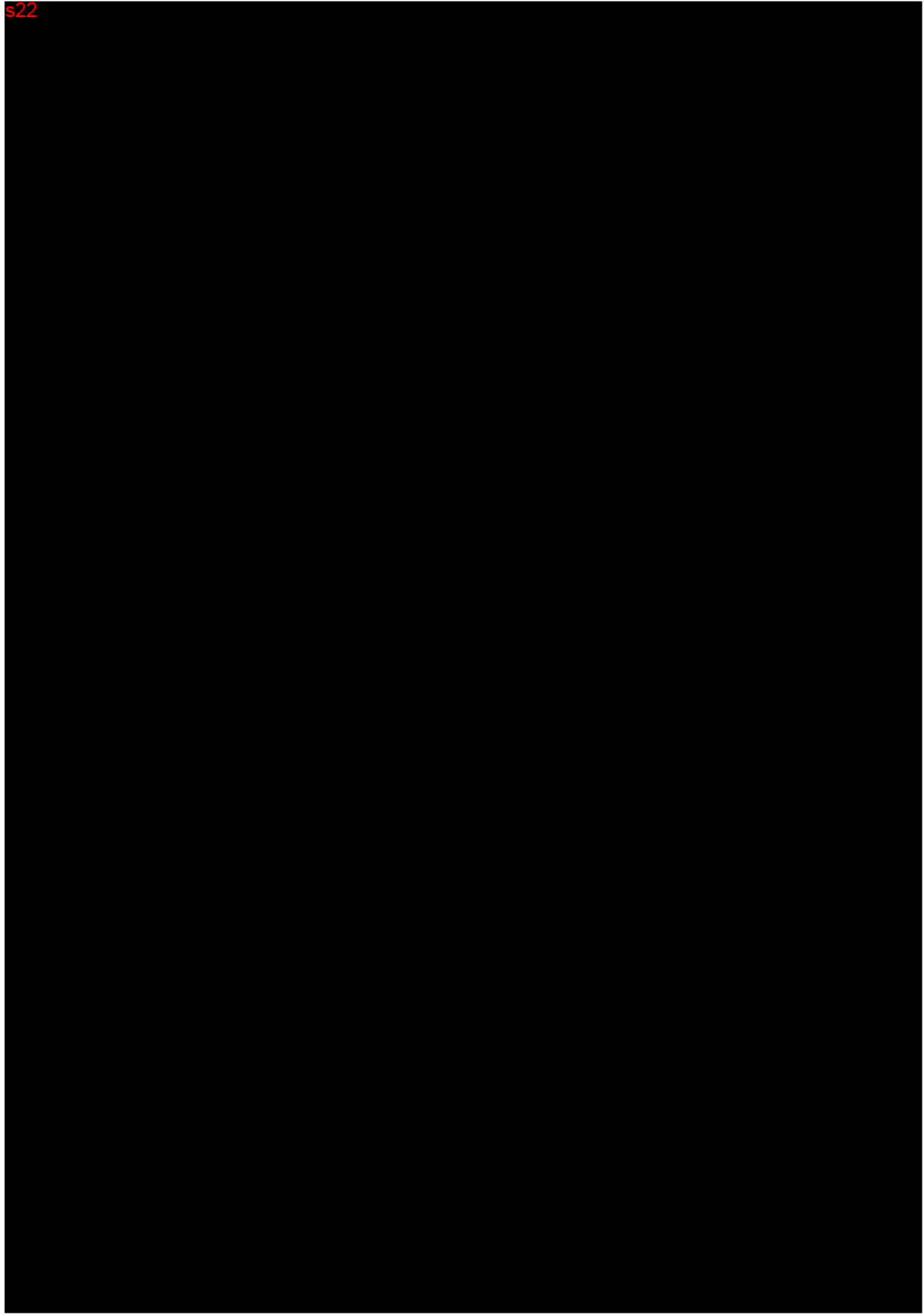




s22



S22

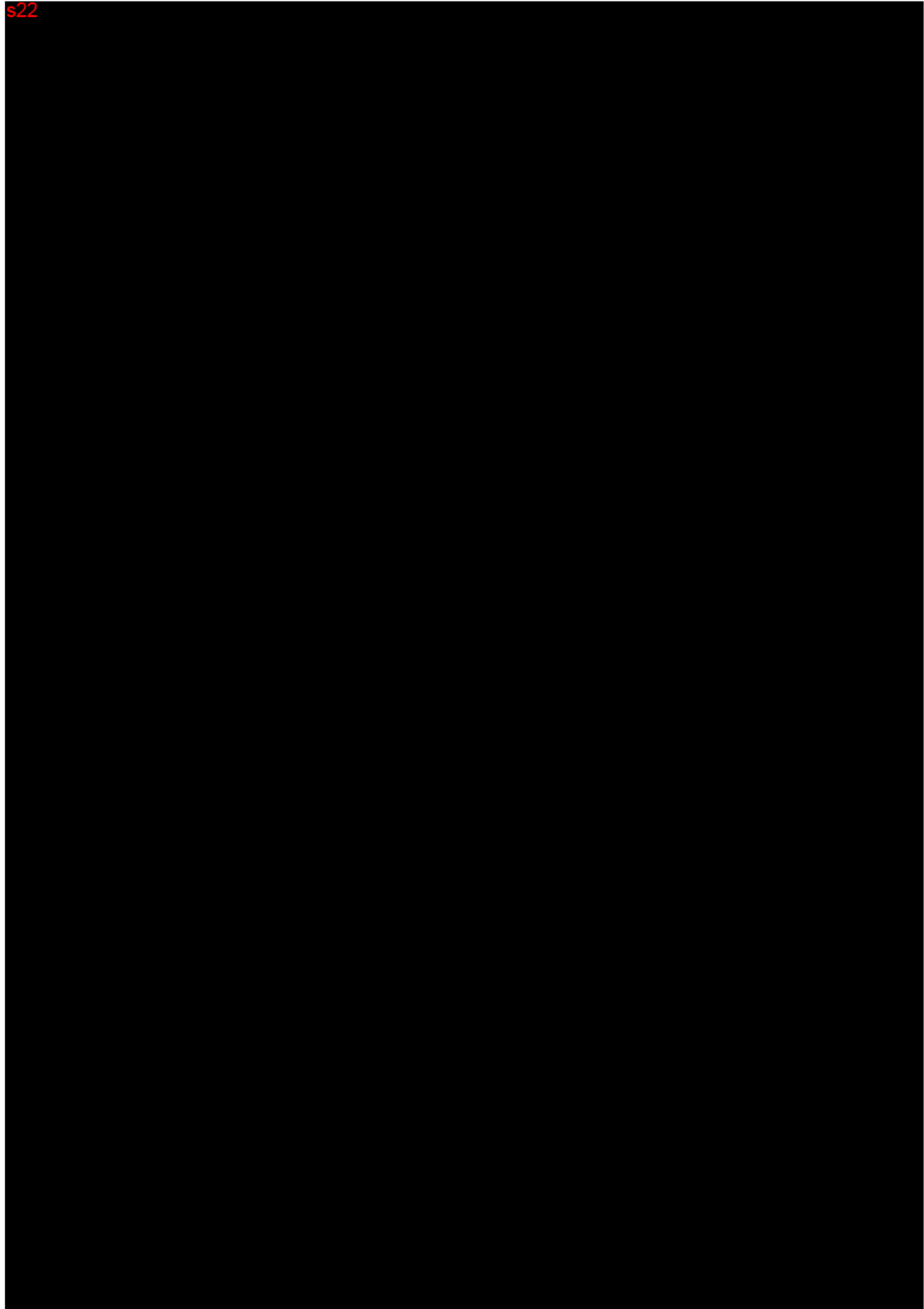


Sample 8					
Plate location (wells)	A8 B8 C8				
LIMS #	2109003420				
BATCH #	000016A				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
% RSD of the % Total for the main peak smear range	≤5%.	4.4			PASS
Peak height	5000-60,000RFU	OK 10000-20000 RFU			PASS
Main peak fragment size (nt)	Comparable to RM	OK ~4000 nt			PASS
Lower Marker	Must be present	OK			PASS
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Main Peak	s47	s47			PASS
Impurity group 1 – RNA fragments					PASS
Impurity group 2 – Poly A tail variant					PASS
Impurity group 3 – RNA lipid adduct					PASS
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
PASS					
Analysist			[Redacted]		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
20 uL of 200 ng/uL + 40 uL Tx100/30% ethanol = 60 uL of 67 ng/uL					

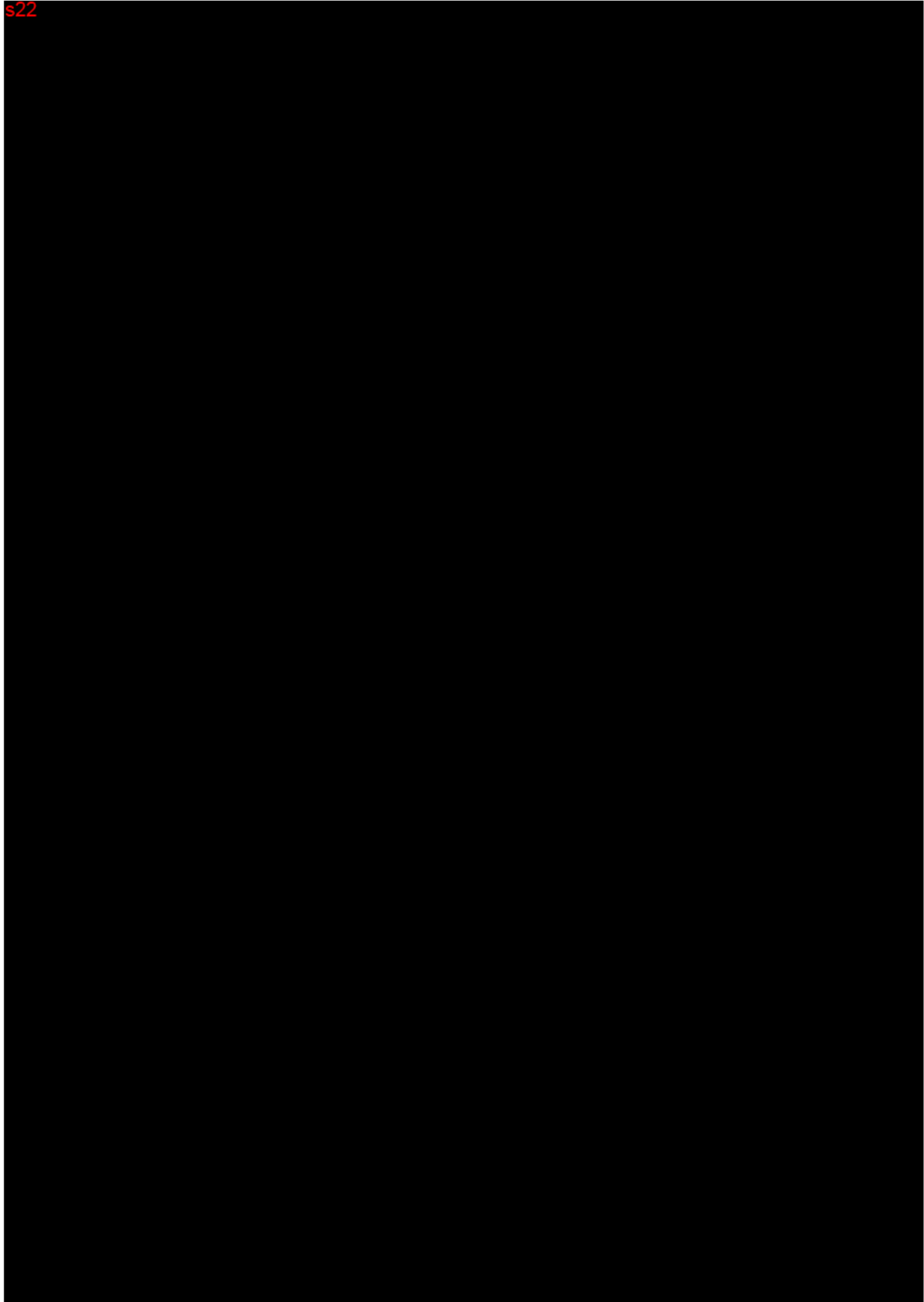
Sample 9					
Plate location (wells)	A9 B9 C9				
LIMS #	2109003363				
BATCH #	3005842				
EXPIRY	Enter date.				
Sample Acceptance Criteria					
Parameter	Limits	Results			Comments
% RSD of the % Total for the main peak smear range	≤5%.	0.8			PASS
Peak height	5000-60,000RFU	OK ~15000 RFU			PASS
Main peak fragment size (nt)	Comparable to RM	OK ~ 4000 nt			PASS
Lower Marker	Must be present	OK			PASS
Enter text.	Enter text.	Enter text.			Choose an item.
Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
Main Peak	s47	s47			PASS
Impurity group 1 – RNA fragments					PASS
Impurity group 2 – Poly A tail variant					PASS
Impurity group 3 – RNA lipid adduct					PASS
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Enter text.	Enter text.	Enter text.	Enter text.	Enter text.	Choose an item.
Sample Results					
PASS					
Analysist			[Redacted]		
Checked by			Enter text.		
Sample Dilutions / Calculation / Notes					
20 uL of 200 ng/uL + 40 uL Tx100/30% ethanol = 60 uL of 67 ng/uL					



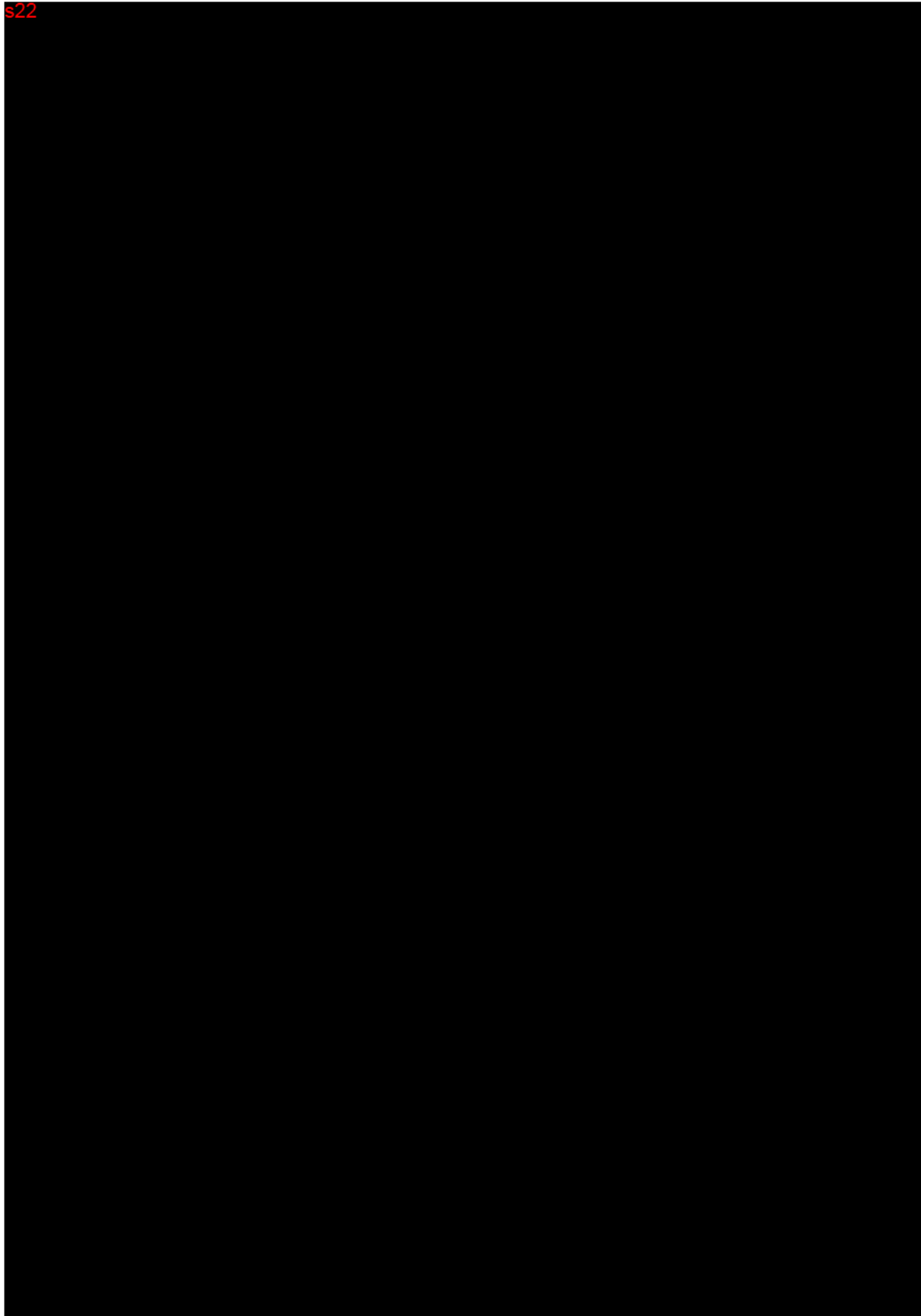
s22



s22



S22





s22



Notes

Enter text.



<b>Owner:</b> s22	<b>Number:</b> Bio-BEE-Form-42
<b>Author:</b> s22	<b>Version:</b> 1
<b>Active:</b> 2/07/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 22Oct2021

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000830072LRW Expiry: 1 March 2021Other Reagent: Pyrospense Batch# 0000904583  
November 2021Expiry: 29 June 2022 Use By: 19

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Moderna Covid vaccine</u>	<u>Q00062A</u>	<u>5 May 2022</u>	<u>2110003933</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
Click or tap here to enter text.	s47	<u>2000</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do:</u> <u>1/50</u>	<u>20uL of Vaccine</u>	<u>975uL</u>	<u>5uL Pyrospense</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrospense</u>
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=
<u>n/a</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>N/A (Undefined)</u>	s47	<u>4.14</u>	<u>146</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

**Notes:**

**Sample appears translucent, white solution, very homogeneous and free from particulates**

Checked **s22** 22Oct2021



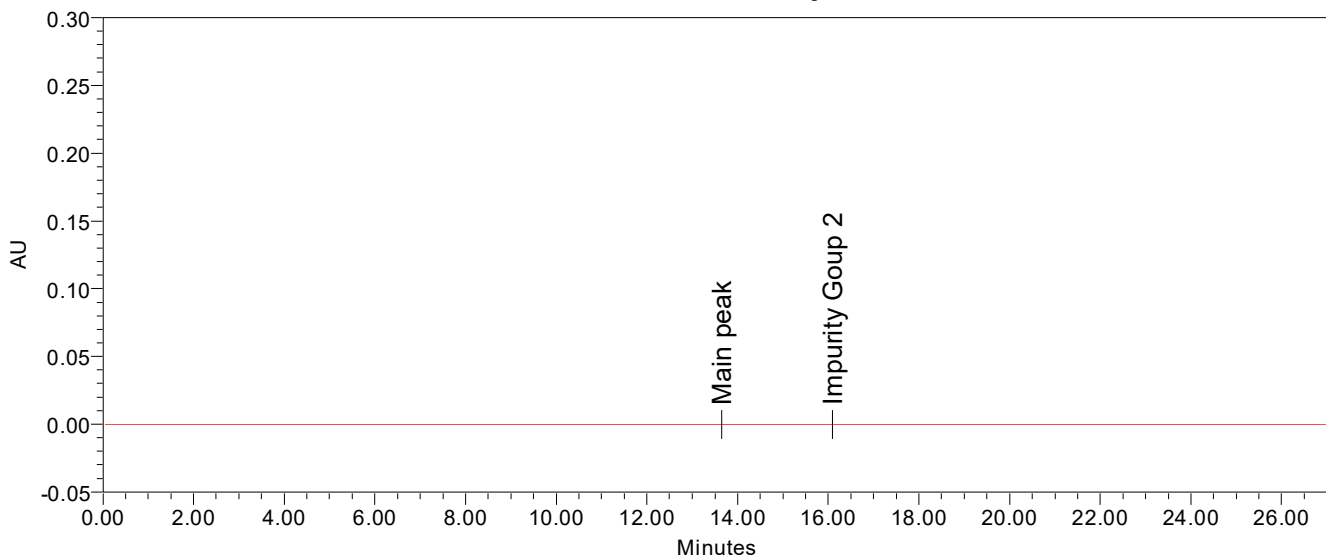
Australian Government  
Department of Health and Ageing  
Therapeutic Goods Administration

Reference Standard, Water Blank, Conditioning,  
2109003363-P1, Sensitivity Solution, 2109003363-P2

Sample Set Name: Spikevax RPIP\_20Sep21\_TK  
Sample Set Acquired By: s22

## System Suitability

Water blank - Prior to sensitivity standard

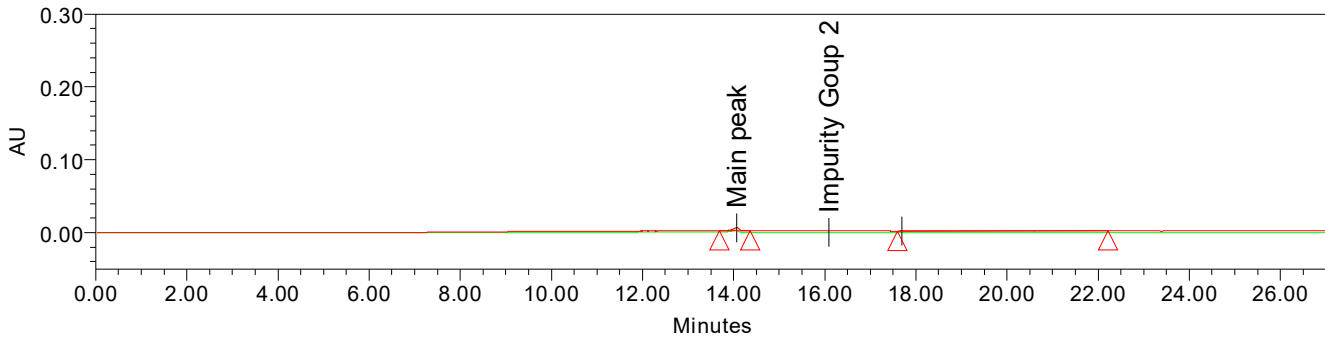


— Label B2; SampleName: Water Blank; Vial: 1:A,1; Injection: 1

Component Results

	Name	RT	Area	Height	Amount	Units	Total Area	TA_Ratio
1	Impurity Group 3						73485	
2	Impurity Group 1						73485	
3	Main peak	14.068	45175	4698			73485	0.63
4	Impurity Goup 2	16.098					73485	

Water blanks - carryover assessment



— Label B3; SampleName: Water Blank; Injection: 1; Channel PDA Ch1 260nm@4.8nm  
 — Label B4; SampleName: Water Blank; Injection: 1; Channel PDA Ch1 260nm@4.8nm

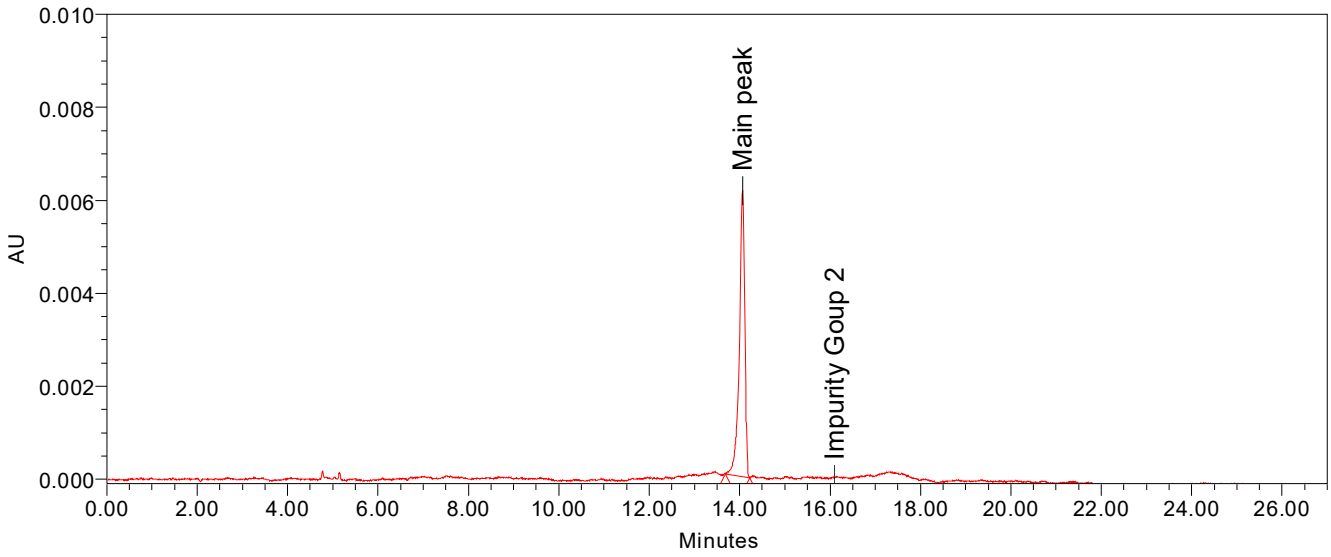
TA\_Ratio Summarized by Name  
 Label: B3

	Label	SampleName	Inj	Total Area	Main peak	Impurity Group 3
1	B3	Water Blank	1	73485	0.63	
Mean				73485		
Std. Dev.						
% RSD						

TA\_Ratio Summarized by Name  
 Label: B4

	Label	SampleName	Inj	Total Area	Main peak	Impurity Group 3
1	B4	Water Blank	1	126797	1.08	1.08
Mean				126797		
Std. Dev.						
% RSD						

Sensitivity Solution

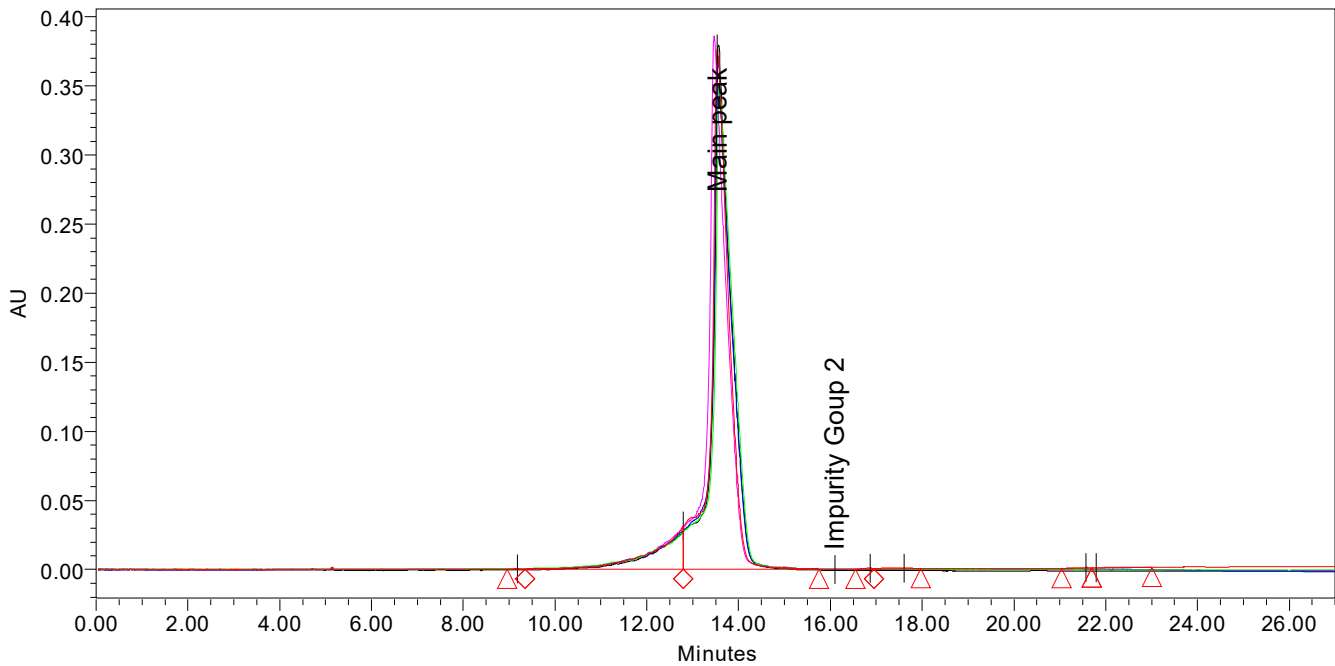


SampleName: Sensitivity Solution; Vial: 1:A,2; Injection: 1; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm

Sensitivity Solution

Name	RT	USP Tailing	USP s/n	Area (μV*sec)	EP s/n	Total Area	TA_Ratio
1 Main peak	14.063	0.778056	284.1	50175	284.1	50175	0.43

First five reference standard injections



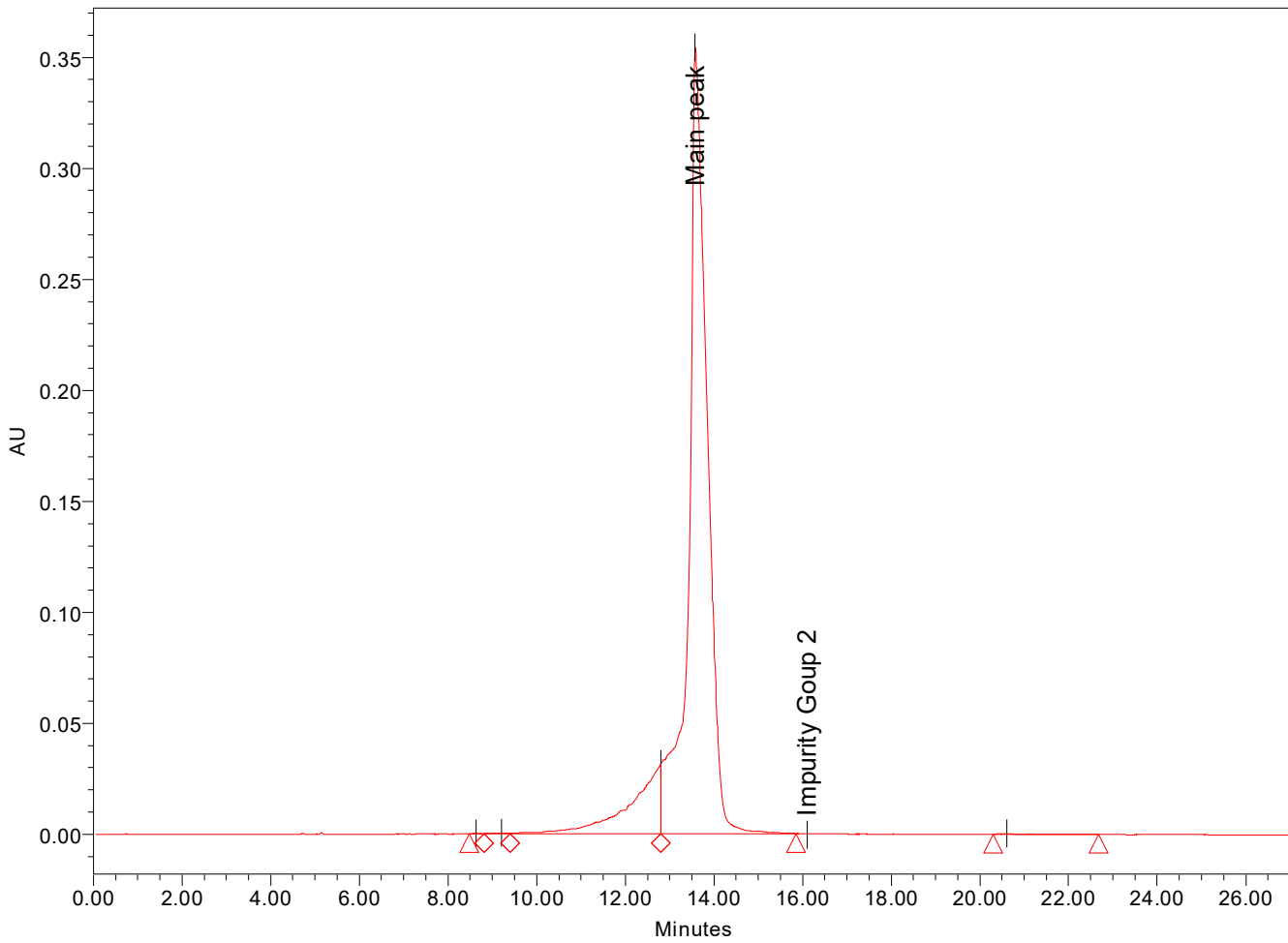
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 1; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 2; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 3; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 4; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm
- SampleName: Reference Standard; Vial: 1:A,3; Injection: 5; Channel Description PDA Ch1 260nm@4.8nm; Channel PDA Ch1 260nm@4.8nm

Component Summary Table  
Name: Main peak

	Sample Name	Vial	Inj	Channel	RT	Area	Total Area
1	Reference Standard	1:A,3	1	PDA Ch1 260nm@4.8nm	13.531	9939743	11322231
2	Reference Standard	1:A,3	2	PDA Ch1 260nm@4.8nm	13.580	10303913	11639618
3	Reference Standard	1:A,3	3	PDA Ch1 260nm@4.8nm	13.544	10531453	11885455
4	Reference Standard	1:A,3	4	PDA Ch1 260nm@4.8nm	13.561	10565707	11886836
5	Reference Standard	1:A,3	5	PDA Ch1 260nm@4.8nm	13.463	10555477	11974202
Mean					13.536	10379259	11741668
Std. Dev.					0.045	268249	265594.8
% RSD					0.3	2.6	2.3



Bracketing Reference Standard



— SampleName: Reference Standard; Vial: 1:A,3; Injection: 1; Label R3

Component Summary Table  
Name: Main peak

	Sample Name	Vial	Inj	Channel	RT	Area	Total Area	Area_Ratio	RT_Ratio
1	Reference Standard	1:A,3	1	PDA Ch1 260nm@4.8nm	13.576	10287834	11759931	99.1	100.3
Mean					13.576	10287834	11759931		
Std. Dev.									
% RSD									

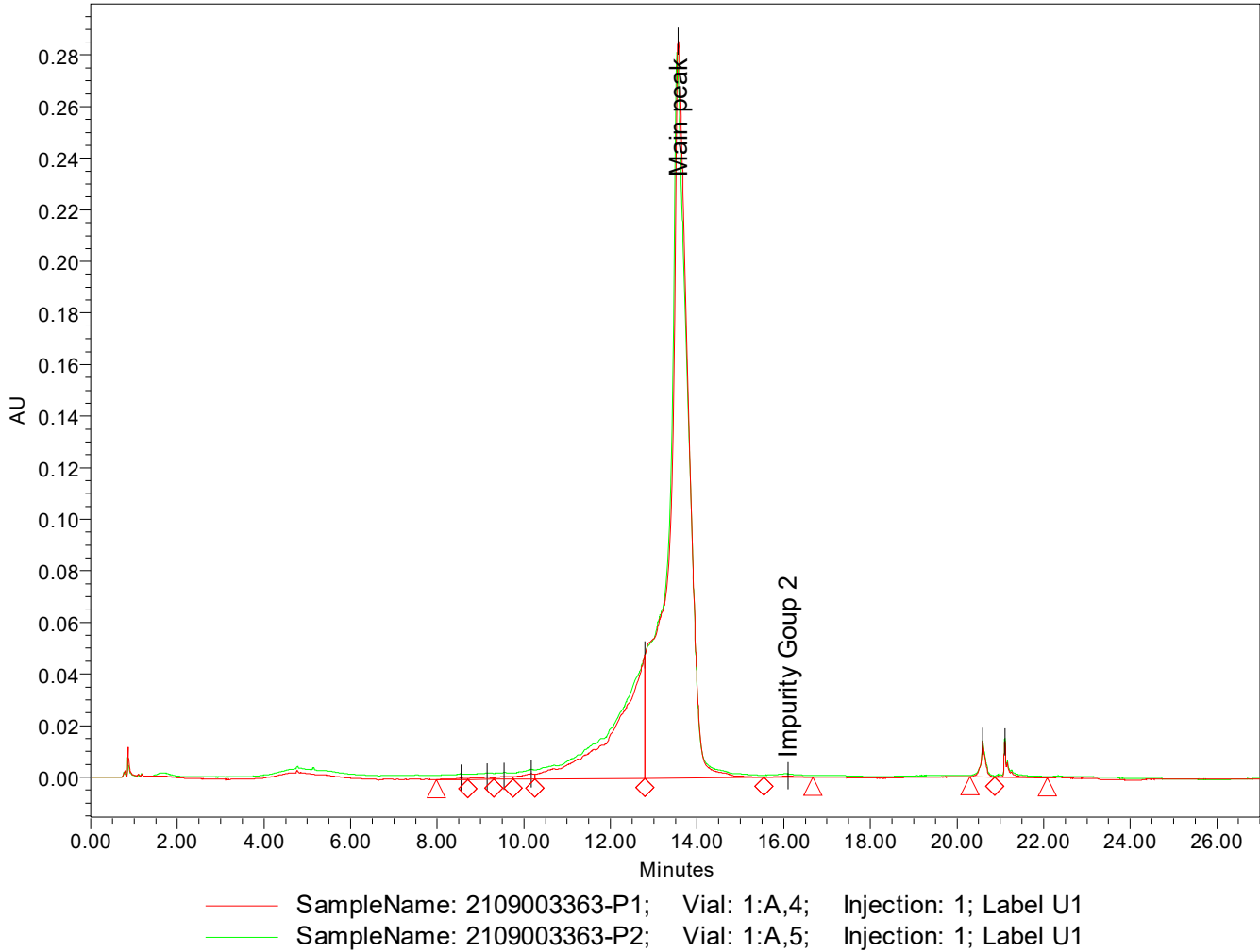
## Sample Acceptance Criteria

TA\_Ratio Summarized by Name  
Label: U1

	Label	SampleName	Inj	Total Area	Impurity Group 1	Main peak	Impurity Goup 2	Impurity Group 3
1	U1	2109003363-P1	1	11072649	94.30	94.30	94.30	94.30
2	U1	2109003363-P2	1	11193631	95.33	95.33	95.33	95.33
Mean				11133140				
Std. Dev.				85547				
% RSD				0.8				

# Sample Results

Sample 1



% Area Summarized by Name  
Label: U1

	Label	SampleName	Inj	Total Area	Impurity Group 1	Main peak	Impurity Goup 2	Impurity Group 3
1	U1	2109003363-P1	1	11072649	S47			
2	U1	2109003363-P2	1	11193631				
Mean				11133140				
Std. Dev.				85547				
% RSD				0.8				







---

Error Log

Overlay Chromatogram group contains information that doesn't match the data being reported.  
Amount Component Summary group contains information that doesn't match the data being reported.  
Overlay Chromatogram group contains information that doesn't match the data being reported.  
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Amount Component Summary group contains information that doesn't match the data being reported.



**Australian Government**  
**Department of Health**  
**Therapeutic Goods Administration**

Laboratories Branch

Operations: HPLC Manual	
Procedure	HPLC – 01 – General HPLC – WORKSHEET
Written	s22
Authorised	
Date issued	12/4/2019
Revision #	8

**HPLC – 01 – General HPLC – WORKSHEET**

TEST DETAILS			
TEST NAME	Analysis of mRNA purity by Size-based RPIP HPLC		
METHOD REFERENCE	Number: SOP-0996 Version: 2.0 Effective Date: 08 Apr 2021		
METHOD MODIFICATIONS (if any)	Waters Acquity system was used, instead of Thermo Vanquish system		
MODIFICATIONS APPROVED BY:	s22		
NAME OF ANALYST	s22	TEST DATE	21-Sep-2021

BUFFERS AND SOLUTIONS	
SOLUTIONS	BATCH No:
MOBILE PHASE A	SW-Mob Ph A-13Sep21
MOBILE PHASE B	SW-Mob Ph B-13Sep21
SAMPLE DILUENT	SW-Dil -13Sep21



PIPETTES USED AND EXPIRY DATES
32833 - Exp: 28/10/21 32891 Exp: 10/12/21 32892 Exp: 24/11/21 32837 Exp: 24/11/21

REFERENCE MATERIALS	
NAME AND CODE	BATCH NO:
mRNA-1273 (2019-nCoV) 2107002766	DH-03180.1

REFERENCE MATERIAL PREPARATIONS AND CALCULATIONS
Reference material was prepared as per SOP

INTERNAL USE ONLY

IN CONFIDENCE

SYSTEM SUITABILITY CRITERIA AND RESULTS			
PARAMETERS	LIMITS	RESULTS	COMMENTS
No peak in the diluent injection (blank) prior to Sensitivity Solution	No peak within assessment window	No peak	Pass
Bracketing blanks must not have interfering peaks more than 1% area of average of reference standards	Interfering peak must be $\leq 1\%$ mean peak area in SST	0.63% 0.76%	Second blank reprocessed manually - D21-3114996
Signal to noise ratio of Sensitivity Solution	$>10$	284	Pass
%RSD of main peak area in first five injections of reference standard	$\leq 5\%$	2.6%	Pass
%RSD of main peak retention time in first five injections of reference standard	$\leq 5\%$	0.3%	Pass
% Recovery of main peak area in bracketing standard(s) compared to average peak area of first five injections of reference standard	95-105%	99.1%	Pass
%Agreement of retention time of main peak from bracketing standard(s) compared to average Retention time of first five injections of reference standard	95-105%	100.3%	Pass
Absolute Difference of main peak area % for duplicate sample preparations	$<5\%$	0.7%	77.6- 76.8=0.8% Pass
Total Sample peak area % recovery for each replicate when compared to average total peak area of first five injections of reference standard	70-130%	94.3% 95.3%	

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME	SPIKEVAX elasomeran 0.2 mg/mL suspension for injection vial		
LIMS No:	2109003363		
BATCH No:	3005842	EXPIRY:	

**SAMPLE DILUTIONS, CALCULATIONS and DATA**

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
0.2mg/mL	-	-	0.5mg/mL	-	10µL

Prepared according to the SOP

**DATA LOCATIONS**

Copies of Empower reports attached?

Data location in TRIM

**TEST RESULTS**

PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS
Impurity Group 1	s47		
Main Peak Area			
Impurity Group 2			
Impurity Group 3			
Total impurities			

SAMPLE RESULTS: Pass

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

DATA LOCATIONS		
Copies of Empower reports attached?		Data location in TRIM

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

DATA LOCATIONS		
Copies of Empower reports attached?	Data location in TRIM	

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

**SAMPLE DILUTIONS, CALCULATIONS and DATA**

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

**DATA LOCATIONS**

Copies of Empower reports attached?	Data location in TRIM
-------------------------------------	-----------------------

**TEST RESULTS**

PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**Comments**



Sample Set Summary Report

Sample Set: Spikevax RPIP\_20Sep21\_TK

Sample Set Information

Project Name: Biochemistry\2021\Moderna RP-IP HPLC

Sample Set Name: Spikevax RPIP\_20Sep21\_TK

SampleName: Reference Standard, Water Blank, Conditioning, 2109003363-P1, Sensitivity Solution, 2109003363-P2

Sample Set Acquired By: s22

Start Date: 20/09/2021 2:04:37 PM AEST

Finish Date: 20/09/2021 10:58:12 PM AEST

Acq Method Set: Moderna RP IP

Run Time: 27.00 Minutes

Instrument Method Name: Moderna RP IP

Sample Set Altered: No

Sample Set Method: Moderna RP IP SSM v2

System Information

System Name: System 4 PDA Only

Analytical\_Column\_1: ID# 500 IonPac AS11-HC

Empower Node: Ucdp191prk2

Analytical\_Column\_2:

Processing Information

Processing Method: Moderna RP IP\_HPLC

Result Set Name: Spikevax RPIP\_20Sep21\_TK

Processed By: s22/Biochem

Result Set Date: 21/09/2021 11:13:54 AM AEST

Processing Method Id: 2866

Result Set Id: 2867

Date Processed: 21/09/2021 11:13:54 AM AEST,  
21/09/2021 11:13:54 AM AEST, 21/09/2021

Channel Description: PDA Ch1 260nm@4.8nm

Processing Node: Uclpdbnjin13

Reporting Information

Report Method Name: Sample Set Summary Report

Print Date: 21/09/2021

Reported by: s22

Time: 11:34:50 AM Australia/Canberra



## Injection Sequence Summary

	SampleName	Sample Type	Vial	Inj #	Run Time (Minutes)	Injection Volume (ul)	Sample Weight	Dilution	Level	Label
1	Conditioning	Control	1:A,3	1	27.00	10.00	1.00000	1.00000		
2	Conditioning	Control	1:A,3	2	27.00	10.00	1.00000	1.00000		
3	Conditioning	Control	1:A,3	3	27.00	10.00	1.00000	1.00000		
4	Water Blank	Control	1:A,1	1	27.00	10.00	1.00000	1.00000		B11
5	Water Blank	Control	1:A,1	1	27.00	10.00	1.00000	1.00000		B12
6	Water Blank	Control	1:A,1	1	27.00	10.00	1.00000	1.00000		B13
7	Water Blank	Control	1:A,1	1	27.00	10.00	1.00000	1.00000		B14
8	Water Blank	Control	1:A,1	1	27.00	10.00	1.00000	1.00000		B2
9	Sensitivity Solution	Standard	1:A,2	1	27.00	10.00	1.00000	1.00000		S
10	Reference Standard	Standard	1:A,3	1	27.00	10.00	1.00000	1.00000		R1
11	Reference Standard	Standard	1:A,3	2	27.00	10.00	1.00000	1.00000		R1
12	Reference Standard	Standard	1:A,3	3	27.00	10.00	1.00000	1.00000		R1
13	Reference Standard	Standard	1:A,3	4	27.00	10.00	1.00000	1.00000		R1
14	Reference Standard	Standard	1:A,3	5	27.00	10.00	1.00000	1.00000		R1
15	Water Blank	Control	1:A,1	1	27.00	10.00	1.00000	1.00000		B3
16	2109003363-P1	Unknown	1:A,4	1	27.00	10.00	1.00000	1.00000		U1
17	2109003363-P2	Unknown	1:A,5	1	27.00	10.00	1.00000	1.00000		U1
18	Water Blank	Control	1:A,1	1	27.00	10.00	1.00000	1.00000		B4
19	Reference Standard	Control	1:A,3	1	27.00	10.00	1.00000	1.00000		R3



Australian Government  
Department of Health and Aged Care  
Therapeutic Goods Administration

Laboratories Branch

Owner: s22	Number: Bio-BEE-Form-42
Author: s22	Version: 1
Active: 2/07/2021	Review: <QPulse_DocReviewDate>
Title: Bacterial Endotoxin - Appendix 1K - Kinetic LAL Routine Assay Sample Results	

# Appendix 1K - Kinetic LAL Routine Assay Sample Results Sheet

Assay ID: 20Sep2021

Operator: s22

LAL Reagent Water (LRW) Lot Number: 0000837899 LRW Expiry: 18 March 2022

Other Reagent: Pyrospense Batch# 0000904583 Expiry: 29 June 2022  
Use By: 20 September 2021

## 1 - Product Details

Product Name	Batch Number	Expiry	LIMS Number
<u>Moderna Covid Vaccine</u>	<u>3005842</u>	<u>3 March 2022</u>	<u>2109003363-R1</u>
Product Concentration	Endotoxin Limit	MVD	Test Dilution
=	s47	<u>2000</u>	<u>1/1000</u>

## 2 - Product Dilutions

Dilution	Volume of Dilution	Volume of LRW	Volume of Other
<u>For 1/1000 do: 1/50</u>	<u>20uL of vaccine</u>	<u>975uL</u>	<u>5uL Pyrospense</u>
<u>1/20</u>	<u>50uL of 1/50</u>	<u>945uL</u>	<u>5uL Pyrospense</u>
<u>N/A</u>	=	=	=
<u>N/A</u>	=	=	=
<u>N/A</u>	=	=	=

## Recording of Results

The results are recorded by the WinKQCL software. Transcribe results from the WinKQCL report to assay sheet.

Test CV (%)	Result (EU/ml)	PPC CV (%)	% PPC Recovery
<u>N/A (Undefined)</u>	s47	<u>3.31</u>	<u>162</u>

For the sample test to be valid, the PPC recovery must be 50-200% of the expected value and the coefficient of variation (CV) of the sample and PPC duplicates should be < 10%. Many samples do not reach an endpoint and are not assigned a %CV. In these instances, contact the person in charge of the assay.

Validity Criteria	
Were the standard curve validity criteria met?	Yes
Were the sample validity criteria met?	Yes
Was the result within the endotoxin limit?	Yes

**This Appendix is used for recording the sample results. Use the SOP (Appendix 8K) for the detailed method.**

**Notes:**

**Sample appears translucent, white solution, very homogeneous and free from particulates**

**Checked s22 20Sep2021 & 12Jan2023**



Australian Government

Department of Health and Ageing  
Therapeutic Goods Administration

## Spikevax - 2209003070, mRNA Std B, Conditioning , Conditioning H2O, Water blank SST, Water blank, Sensitivity. mRNA Std A. Water blank

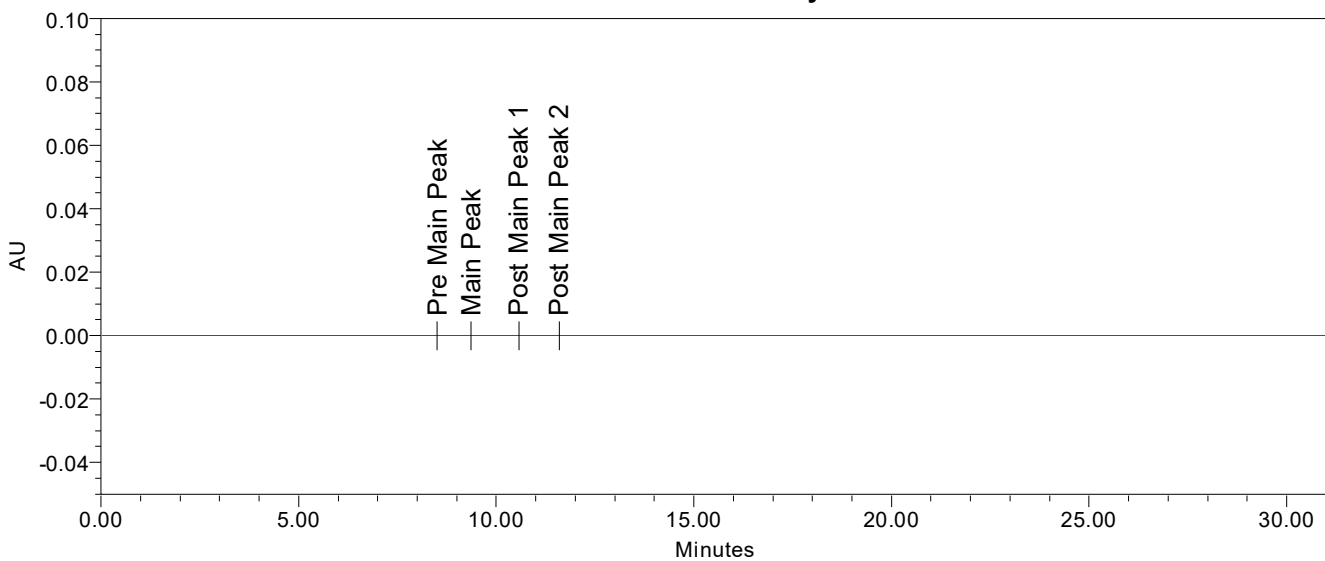
Sample Set Name: Spikevax

RPIP\_Paediat\_27Sep22

Sample Set Acquired By: s22

### System Suitability

Water blank - Prior to sensitivity standard

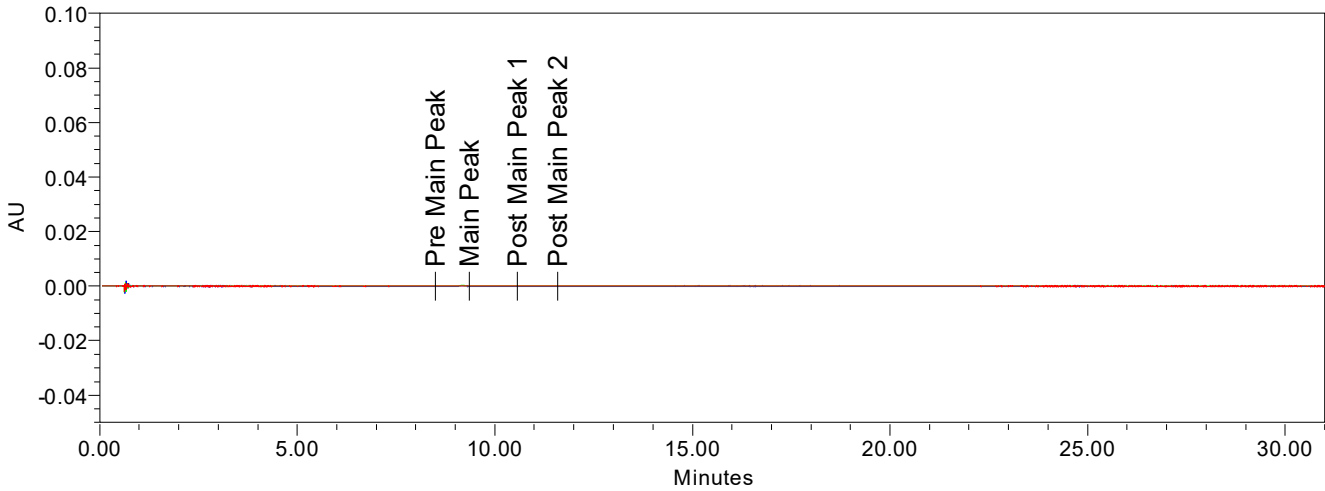


— Label B1; SampleName: Water blank; Vial: 1:E,2; Injection: 1

Component Results

	SampleName	Name	RT	Height	% Area	AreaRatio	Area (μV*sec)
1	Water blank	IG: RNA Fragments					
2	Water blank	IG: Lipid adducts					
3	Water blank	Pre Main Peak	8.500				
4	Water blank	Main Peak	9.357				
5	Water blank	Post Main Peak 1	10.570				
6	Water blank	Post Main Peak 2	11.590				

Water blank - Carryover assessment



- Label B0; SampleName: Water blank SST; Vial: 1:E,2; Injection: 1
- Label B2; SampleName: Water blank; Vial: 1:E,2; Injection: 1
- Label B2; SampleName: Water blank; Vial: 1:E,2; Injection: 1

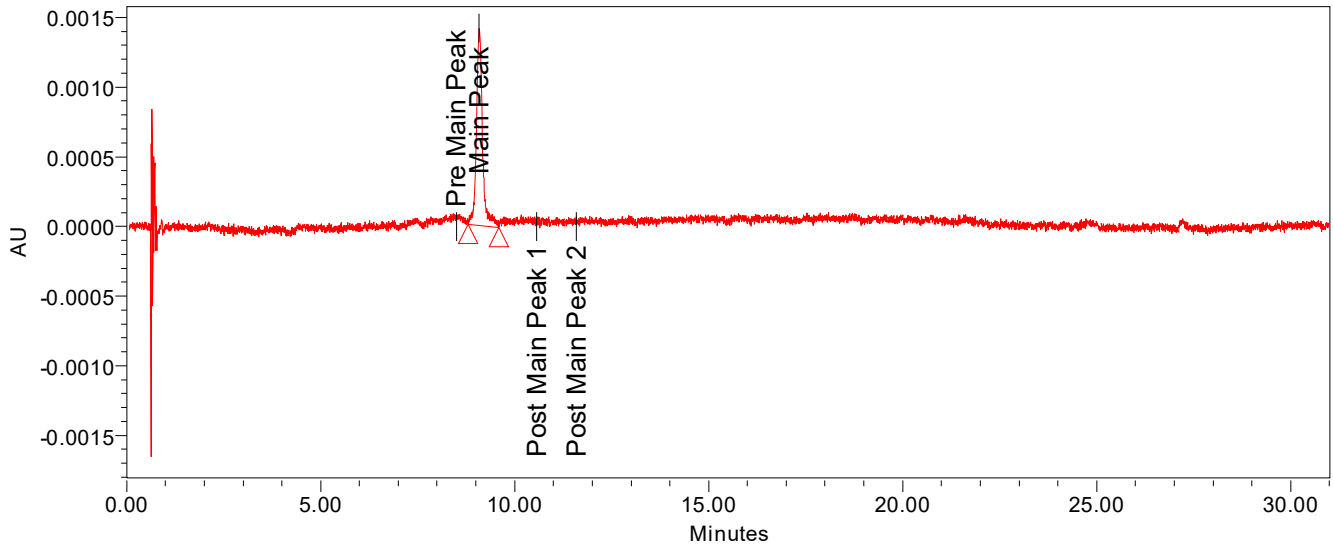
Component Results  
AreaRatio Summarized by Name

	SampleName	Label	IG: RNA Fragments	IG: Lipid adducts	Pre Main Peak	Main Peak
1	Water blank SST	B0				
2	Water blank	B2				0.1467
3	Water blank	B2				0.1605

Component Results  
AreaRatio Summarized by Name

	Post Main Peak 1	Post Main Peak 2
1		
2		
3		

Sensitivity Solution



SampleName: Sensitivity; Vial: 1:E,3; Injection: 1; Channel Description PDA Ch1 260nm@4.8nm;  
 Channel PDA Ch1 260nm@4.8nm

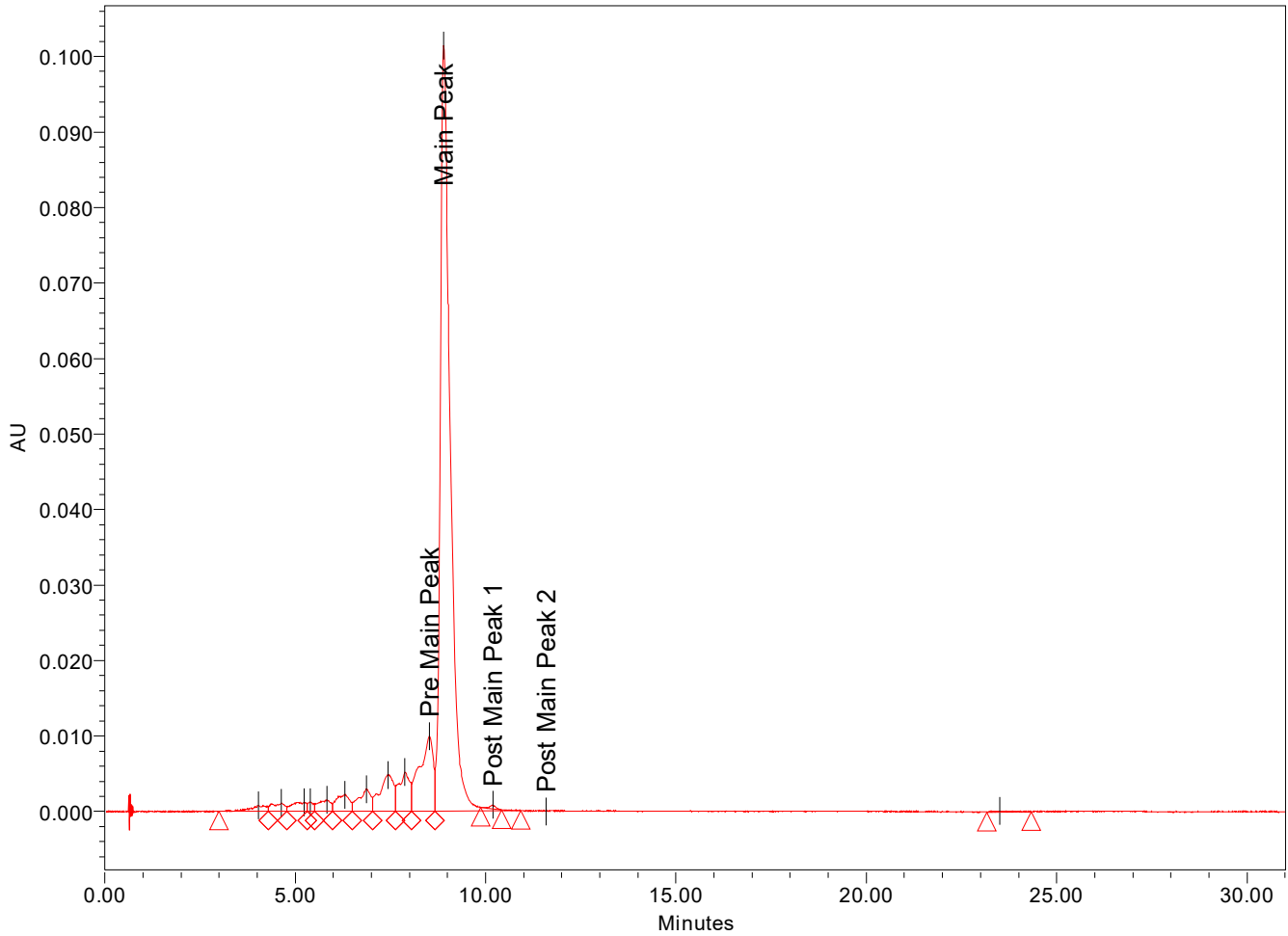
Sensitivity Solution  
 s/n Summarized by Name

	IG: RNA Fragments	IG: Lipid adducts	Pre Main Peak	Main Peak	Post Main Peak 1
1				18.40	

Sensitivity Solution  
 s/n Summarized by  
 Name

	Post Main Peak 2
1	

Bracketing Reference Standard

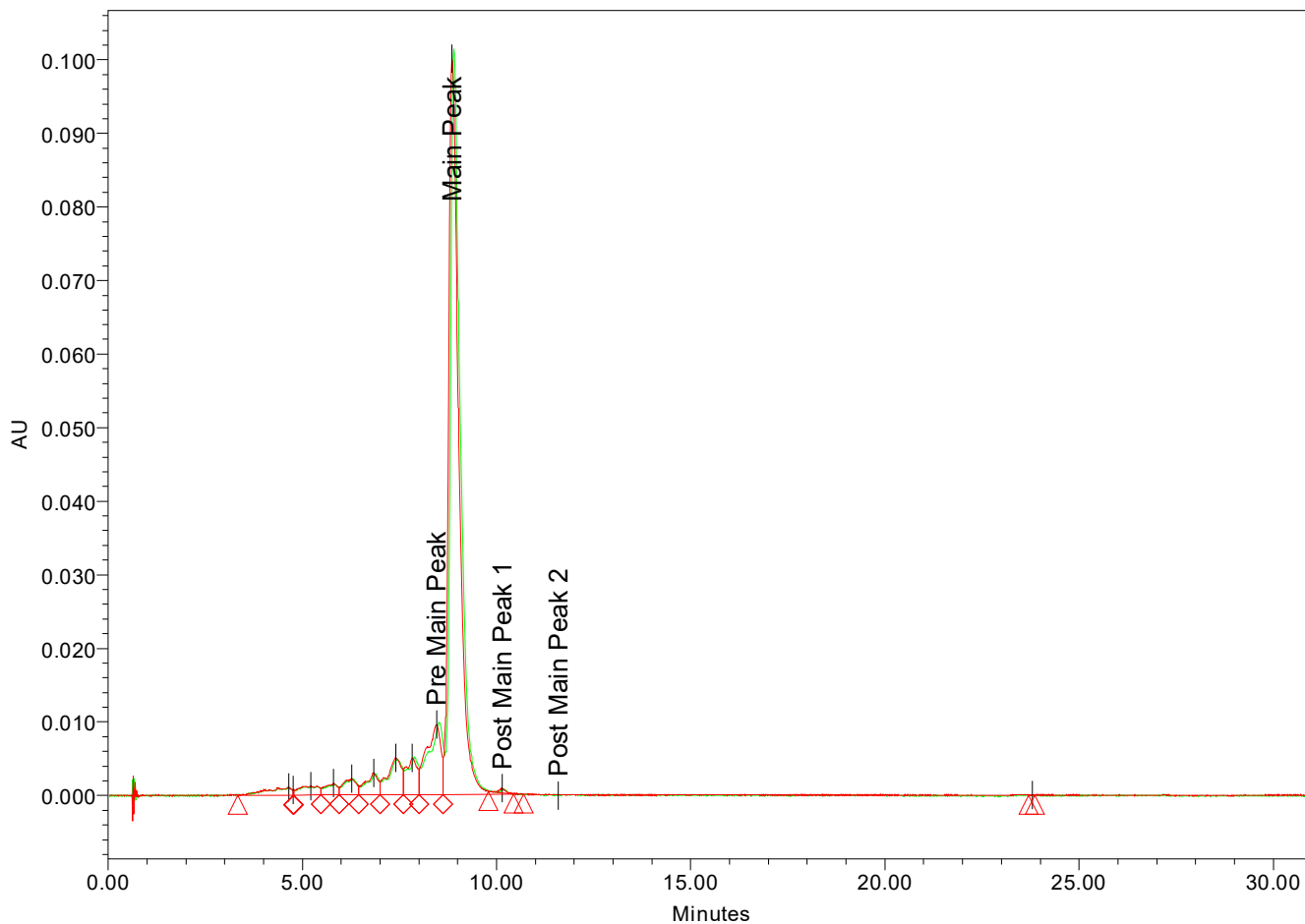


— SampleName: mRNA Std B; Vial: 1:A,3; Injection: 1; Label S0201

Component Summary Table  
Name: Main Peak

Sample Name	Name	Vial	Inj	Total Area	Label	Area (μV*sec)	Std_AreaRatio	Std_RT_ratio
1 mRNA Std B	Main Peak	1:A,3	1	2555671	S0201	1830980	100.4	100.47

Summary of Reference Standard Injections



— SampleName: mRNA Std A; Vial: 1:A,1; Injection: 1; Label S0101  
 — SampleName: mRNA Std B; Vial: 1:A,3; Injection: 1; Label S0201

Component Summary Table  
 Area Summarized by Name

	Sample Name	Inj	Total Area	Label	IG: RNA Fragments ( $\mu\text{V}\cdot\text{sec}$ )	Pre Main Peak ( $\mu\text{V}\cdot\text{sec}$ )	Main Peak ( $\mu\text{V}\cdot\text{sec}$ )	Post Main Peak 1 ( $\mu\text{V}\cdot\text{sec}$ )
1	mRNA Std A	1	2537395	S0101	706691	244486	1823134	7138
2	mRNA Std B	1	2555671	S0201	713386	245673	1830980	5840
Mean			2546533		710038.3	245079.7	1827056.8	6489.3
% RSD			0.5		0.7	0.3	0.3	14.1

Component Summary Table  
 Area Summarized by Name

	IG: Lipid adducts ( $\mu\text{V}\cdot\text{sec}$ )
1	7570
2	11305
Mean	9437.6
% RSD	28.0



Component Summary Table  
Std\_AreaRatio Summarized by Name

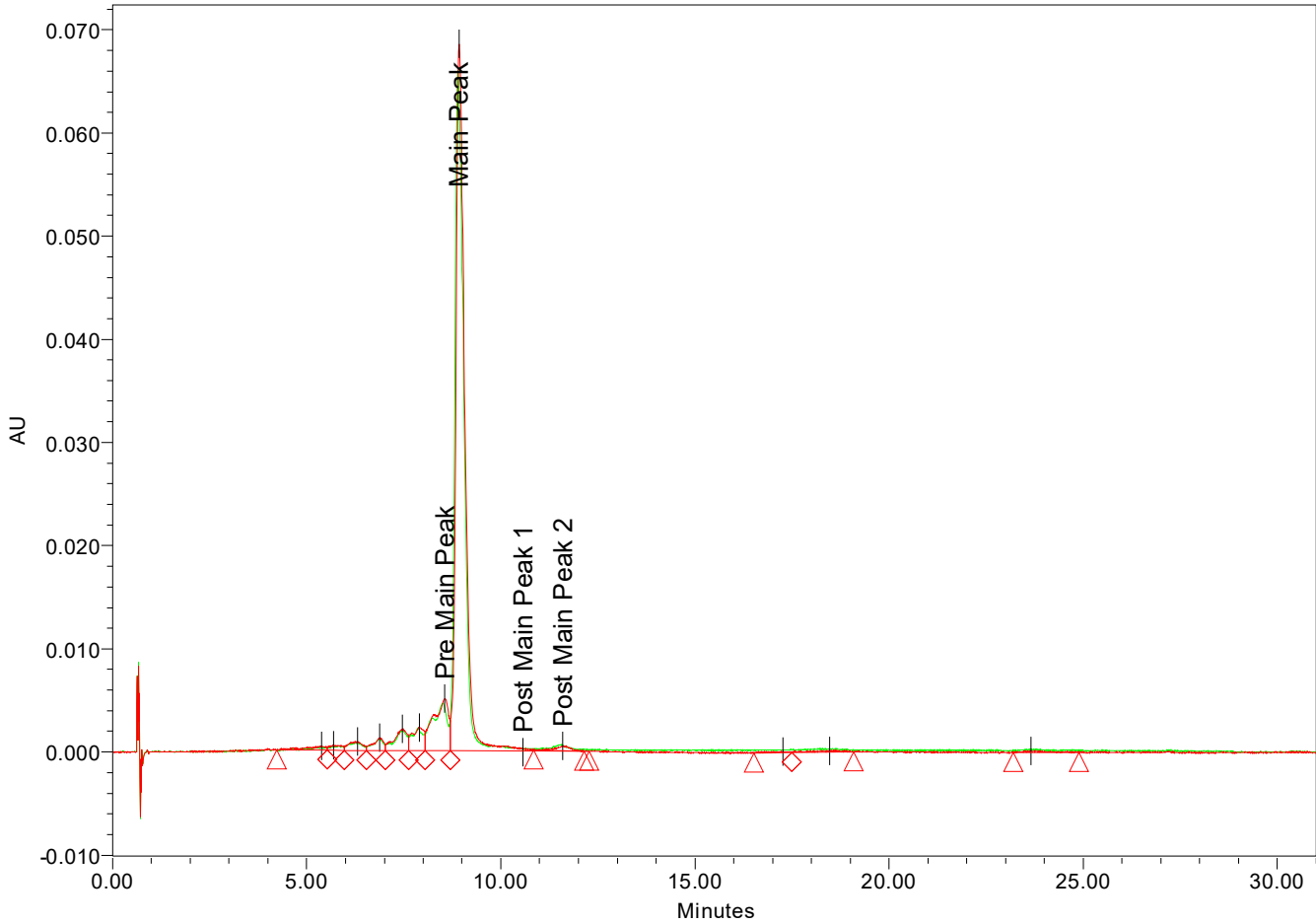
	Sample Name	Inj	Total Area	Label	IG: RNA Fragments	Pre Main Peak	Main Peak	Post Main Peak 1
1	mRNA Std A	1	2537395	S0101	100.0	100.0	100.0	100.0
2	mRNA Std B	1	2555671	S0201	100.9	100.5	100.4	81.8
Mean			2546533		100.5	100.2	100.2	90.9
% RSD			0.5		0.7	0.3	0.3	14.1

Component Summary Table  
Std\_AreaRatio Summarized  
by Name

	IG: Lipid adducts
1	100.0
2	149.3
Mean	124.7
% RSD	28.0

# Sample Results

Sample 1



— SampleName: Spikevax - 2209003070; Vial: 1:A,2; Injection: 1; Label U0101  
 — SampleName: Spikevax - 2209003070; Vial: 1:A,2; Injection: 2; Label U0101

% Area Summarized by Name  
 Label: U0101

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts
1	U0101	Spikevax - 2209003070	1	s47			
2	U0101	Spikevax - 2209003070	2				
Mean							
% RSD							

% Area Summarized by Name  
 Label: U0101

	Post Main Peak 2
1	s47
2	
Mean	
% RSD	

Sample\_AreaDif Summarized by Name  
Label: U0101

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts
1	U0101	Spikevax - 2209003070	2	s47			

Sample\_AreaDif  
Summarized by Name  
Label: U0101

	Post Main Peak 2
1	s22








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Error Log

Overlay Chromatogram group contains information that doesn't match the data being reported.  
Amount Component Summary group contains information that doesn't match the data being reported.  
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**Australian Government**  
**Department of Health**  
Therapeutic Goods Administration

Laboratories Branch

Operations: HPLC Manual	
Procedure	HPLC – 01 – General HPLC – WORKSHEET
Written	s22
Authorised	[Redacted]
Date issued	12/4/2019
Revision #	8

**HPLC – 01 – General HPLC – WORKSHEET**

TEST DETAILS			
TEST NAME	Analysis of mRNA purity in Moderna SpikeVax Paediatric vaccine by Size-based RPIP		
METHOD REFERENCE	Moderna SOP-1142		
METHOD MODIFICATIONS (if any)	None		
MODIFICATIONS APPROVED BY:	N/A		
NAME OF ANALYST	s22	TEST DATE	27-Sep-2022

BUFFERS AND SOLUTIONS	
SOLUTIONS	BATCH No:
MOBILE PHASE A	ST14Sep22-1
MOBILE PHASE B	ST14Sep22-2
SAMPLE DILUENT	



PIPETTES USED AND EXPIRY DATES
LIMS# 32837, Exp: 21/10/22 LIMS#33250, Exp: 07/10/22 LIMS#32677, Exp: 06/10/22

REFERENCE MATERIALS	
NAME AND CODE	BATCH NO:
mRNA Ref. Std, CX-024414, Conc: 3.2mg/ml	RM-4007421130

REFERENCE MATERIAL PREPARATIONS AND CALCULATIONS
Conc. of Ref. Std 3.2mg/ml Required/Working concentration: 0.2mg/ml Stock Ref was diluted to 16x with RNase free water 100ul of stock Ref. Std. was mixed with 1500ul of DEPC water to make 0.2mg/ml  Sensitivity Solution (0.002mg/ml): Taken 10ul of 0.2mg/ml Ref std and then mixed with 990ul RNase free water to make 0.002mg/ml of sensitivity solution.

SYSTEM SUITABILITY CRITERIA AND RESULTS			
PARAMETERS	LIMITS	RESULTS	COMMENTS
No peak in the water blank injection prior to the Sensitivity Solution	No peak	Conform	Pass
All other carryover water blank injections in the sequence interfere with the peaks of interest as compared to the total peak area of the mRNA working reference standard	Interference <=1%	Conform	Pass
Signal to Noise ratio of Sensitivity Solution	>=10	18.40	Pass
% Recovery of the main peak area in each bracketing standard compared to the peak area of the mRNA working reference standard	90-110%	100.4	Pass
% Agreement of the main peak RT in each bracketing standard compared to the main peak RT of the mRNA working reference standard	90-110%	100.47	Pass
Absolute Difference of the Main Peak % Area for duplicate sample preparations.	<=5%	2.858	Pass

INTERNAL USE ONLY

IN CONFIDENCE

SAMPLE DETAILS			
SAMPLE NAME	SPIKEVAX elasomeran 0.1 mg/mL suspension for injection vial		
LIMS No:	2209003070		
BATCH No:	054F22A	EXPIRY:	03-May-2023

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
0.1 mg/ml	-	-	0.1 mg/ml	-	10ul
No dilution required. 100ul of neat sample taken into UPLC total recover vial for injection.					
DATA LOCATIONS					
Copies of Empower reports attached?	Yes	Data location in TRIM	E22-542283		

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS
IG: Fragment	s47		
Main Peak (RNA purity)			
IG: Lipid adducts			

SAMPLE RESULTS: Pass

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

DATA LOCATIONS		
Copies of Empower reports attached?		Data location in TRIM

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

DATA LOCATIONS	
Copies of Empower reports attached?	Data location in TRIM

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

**SAMPLE DILUTIONS, CALCULATIONS and DATA**

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

**DATA LOCATIONS**

Copies of Empower reports attached?	Data location in TRIM
-------------------------------------	-----------------------

**TEST RESULTS**

PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**Comments**



Australian Government

Department of Health and Ageing  
Therapeutic Goods Administration

## Sample Set Summary Report

Sample Set: Spikevax RPIP\_Paediat\_27Sep22

### Sample Set Information

Project Name: Biochemistry\2022\Spikevax RPIP

Sample Set Name: Spikevax RPIP\_Paediat\_27Sep22

Sample Set Acquired By: s22

Start Date: 27/09/2022 2:19:02 PM AEST

Finish Date: 28/09/2022 1:17:59 AM AEST

Run Time: 31.00 Minutes

Sample Set Altered: No

SampleName: Spikevax - 2209003070, mRNA Std B, Conditioning, Conditioning H2O, Water blank SST, Water blank, Sensitivity, mRNA Std A, Water blank

Acq Method Set: Spikevax RPIP\_2022\_MS

Instrument Method Name: Spikevax RPIP\_2022\_IM

Sample Set Method: !QuickSet

### System Information

System Name: System 4 PDA only

Empower Node: Ucdp5n8zsf2

Analytical\_Column\_1: ID#518 ProSwift RP-1S 4.6x50mm Sn002703

Analytical\_Column\_2:

### Processing Information

Processing Method: Spikevax RPIP\_2022\_v3

Processed By: s22/Biochem

Processing Method Id: 6012

Date Processed: 28/09/2022 1:28:06 PM AEST, 28/09/2022 1:28:08 PM AEST, 28/09/2022 1:28:10

Processing Node: Uclpbh7In13

Result Set Name: Spikevax RPIP\_Paediat\_27Sep22

Result Set Date: 28/09/2022 1:28:03 PM AEST

Result Set Id: 6054

Channel Description: PDA Ch1 260nm@4.8nm

### Reporting Information

Report Method Name: Sample Set Summary Report

Print Date: 28/09/2022

Reported by: s22

Time: 1:34:26 PM Australia/ACT



## Injection Sequence Summary

	SampleName	Sample Type	Vial	Inj #	Run Time (Minutes)	Injection Volume (ul)	Sample Weight	Dilution	Level	Label
1	Conditioning	Control	1:E,1	1	31.00	10.00	1.00000	1.00000		
2	Conditioning	Control	1:E,1	2	31.00	10.00	1.00000	1.00000		
3	Conditioning	Control	1:E,1	3	31.00	10.00	1.00000	1.00000		
4	Conditioning	Control	1:E,1	4	31.00	10.00	1.00000	1.00000		
5	Conditioning	Control	1:E,1	5	31.00	10.00	1.00000	1.00000		
6	Conditioning H2O	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
7	Conditioning H2O	Control	1:E,2	2	31.00	10.00	1.00000	1.00000		
8	Conditioning H2O	Control	1:E,2	3	31.00	10.00	1.00000	1.00000		
9	Conditioning H2O	Control	1:E,2	4	31.00	10.00	1.00000	1.00000		
10	Water blank SST	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B0
11	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B1
12	Sensitivity	Control	1:E,3	1	31.00	10.00	1.00000	1.00000		A
13	mRNA Std A	Standard	1:A,1	1	31.00	10.00	1.00000	1.00000		S0101
14	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B2
15	Spikevax - 2209003070	Unknown	1:A,2	1	31.00	10.00	1.00000	1.00000		U0101
16	Spikevax - 2209003070	Unknown	1:A,2	2	31.00	10.00	1.00000	1.00000		U0101
17	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
18	mRNA Std B	Standard	1:A,3	1	31.00	10.00	1.00000	1.00000		S0201
19	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B2



<b>Type:</b> Biotherapeutics\BEE\Forms	<b>Number:</b> Bio-BEE-Form-39 / <b>Version:</b> 3
<b>Owner:</b> s22	<b>Approver:</b> s22
<b>Active:</b> 10/05/2022	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Endotoxin Routine Assay Worksheet	

## Endotoxin Routine Assay Worksheet

Assay ID: 28Sep22 Operator: s22

### Limulus Amoebocyte Lysate (LAL)

Lysate batch and expiry recorded on software for each assay

Ensure sensitivity of LAL batch has been confirmed. 'Lysate preparation details' shown below

### Recombinant Factor C (rFC)

rFC Enzyme, Fluorogenic Substrate & rFC Assay Buffer batches and expiry dates recorded on software for each assay

Ensure sensitivity of the rFC batch has been confirmed. 'rFC Reagent preparation details' shown below

### Control Standard Endotoxin (CSE) – refer to Bio-BEE-Method 5 and Bio-BEE-Form 37

CSE batch and expiry recorded on software for each assay

Reconstitution details for either KLAL or rFC – see Trim File D22-5933959

CSE Lot Number: 0000981165 Conc 50 EU/mL

### LAL Reagent Water (LRW) Lot Number: 00010508447 Expiry: 05Apr2024

How many samples were linked to this assay? 1

**This form is used for recording the assay details and results and only gives the method in point form. See Bio-BEE-SOP 28 and appropriate method for the detailed procedure.**

To avoid endotoxin contamination, use careful technique and **pyrogen free** equipment.

### Preparation of Assay

- Fill out the appropriate forms from the Quality Management System (QMS)
- Retrieve the required kit reagents from cold storage to equilibrate to room temperature
- Turn on plate reader and computer and follow the steps as detailed in Bio-BEE-Method 4

### Preparation of CSE (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare CSE as detailed in Bio-BEE-Method 5. CSE dilutions can be dispensed to the plate as they are prepared to save mixing time

- CSE is set up as in the table below - record %CV results from the final report

#### For a KLAL (KQCL) assay

Concentration	Plate wells ID	% CV
50 EU/ml	F1 – F2	0.79
5 EU/ml	E1 – E2	0.86
0.5 EU/ml	D1 – D2	0.05
0.05 EU/ml	C1 – C2	2.34
0.005 EU/ml	B1 – B2	0.54
Blank	A1 – A2	

#### For an rFC assay

Concentration	Plate wells ID	% CV
5 EU/ml	E1 – E2	-
0.5 EU/ml	D1 – D2	-
0.05 EU/ml	C1 – C2	-
0.005 EU/ml	B1 – B2	-
Blank	A1 – A2	

- Dispense 100 µl of the appropriate dilution of CSE into the appropriate wells of the plate.
- Continue with procedure as per the appropriate method

### Preparation of Samples (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare sample dilutions as in Bio-BEE-Form 42
- Pipette 10 µl of the 5 EU/ml standard to the appropriate PPC wells as per the plate layout
- Dispense 100 µl of the final sample dilutions into the 4 appropriate wells as per the plate layout

### Starting the Assay

- The plate is then ready for the reaction. Prepare the software as set out in the Bio-BEE-Method 4
- “Run” the Template prepared earlier. Follow the prompts to the Pre-warming step

#### If performing a KLAL assay

- Prepare the required lysate vial/s (Bop-BEE-Method 6) and pour into the reagent reservoir

Lysate Preparations Details -

Lysate Lot Number	XL021QDD4P	Expiry 17Aug2023
Date sensitivity confirmed	09May2022	TRIM#: E22-564008
Reconstitute lysate with	2.6mL (2 x 1mL + 600uL)	mL of LAL Reagent Water (LRW)
Date reconstituted	28Sep2022	-
Operator(s)	s22	-
Use by date	12Oct2022	(Lonza KQCL- 14 days from reconstitution, at below -10°C)

**OR**

*If performing an rFC assay*

- Make up the required volume of rFC reagent directly into the reservoir (Bio-BEE-Method 6)

rFC Reagent Preparations Details – (Note: once prepared, working reagent cannot be stored)

	Lot	Expiry	Volume
Date sensitivity confirmed	-	-	
Fluorogenic Substrate	-	--	- µL
rFC Assay Buffer	-	-	- µL
rFC Enzyme Solution	-	-	- µL

- Open cover – if using the Spectramax use the software to open and close the drawer
- Add 100 µl of either lysate (KLAL) **OR** working reagent (rFC) to each of the assay wells, carefully, and as quickly as possible
- Close the drawer on the plate reader and click OK to start the run. **Do not open drawer**

**Acceptance Criteria – for KLAL**

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>-0.998</u>
Slope between -0.400 and -0.100	<u>-0.227</u>
Y intercept between 2.500 and 3.500	<u>3.086</u>
Mean reaction times of blank $\geq$ mean reaction times of lowest standard	<u>Yes</u>
Coefficient of variation (CV) values for all standards are $< 10\%$	<u>Yes</u>
Were all acceptance criteria for the standard curve met?	<u>Yes</u>

## Acceptance Criteria – for rFC

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	=
Slope between 0.760 and 1.110	=
Y intercept between 2.500 and 5.000	=
Mean RFU of blank $\leq$ mean RFU of lowest standard	=
Coefficient of variation (CV) values for all standards are $< 25\%$	=
Were all acceptance criteria for the standard curve met?	=

## Conclusions

Follow procedures for 'Recording Results' detailed in Bio-BEE-Method 7

## Notes

Click or tap here to enter text.



Australian Government

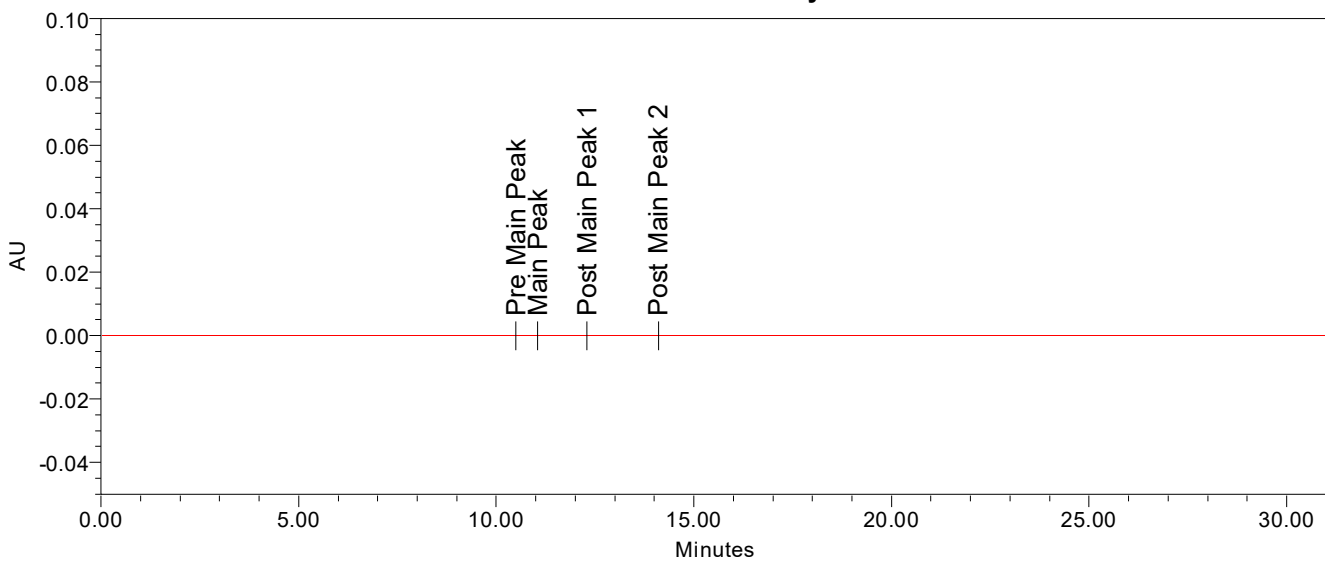
Department of Health and Ageing  
Therapeutic Goods Administration

mRNA Std B, Spikevax Bivalent - 2208002750,  
Conditioning , Conditioning H2O, Water blank SST,  
Water blank. s22

Sample Set Name: Sys  
3\_Bivalent\_NewRPIP\_31Aug22  
Sample Set Acquired By: s22

## System Suitability

Water blank - Prior to sensitivity standard

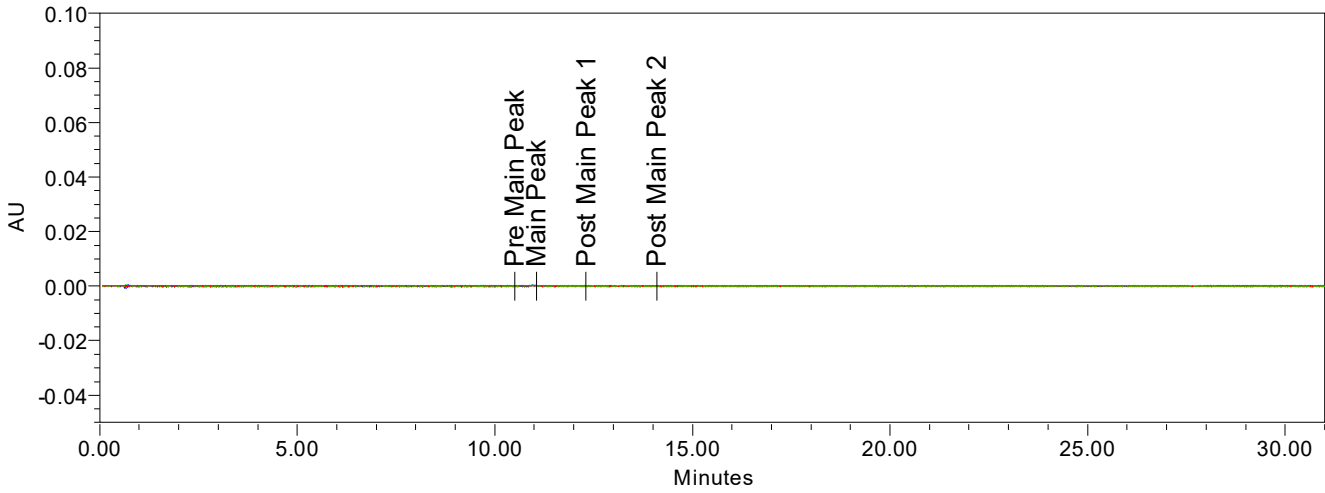


— Label B1; SampleName: Water blank; Vial: 1:E,2; Injection: 1

Component Results

	SampleName	Name	RT	Height	% Area	AreaRatio	Area (μV*sec)
1	Water blank	IG: RNA Fragments					
2	Water blank	IG: Lipid adducts					
3	Water blank	Pre Main Peak	10.500				
4	Water blank	Main Peak	11.050				
5	Water blank	Post Main Peak 1	12.300				
6	Water blank	Post Main Peak 2	14.100				

Water blank - Carryover assessment



- Label B0; SampleName: Water blank SST; Vial: 1:E,2; Injection: 1
- Label B2; SampleName: Water blank; Vial: 1:E,2; Injection: 1
- Label B2; SampleName: Water blank; Vial: 1:E,2; Injection: 1

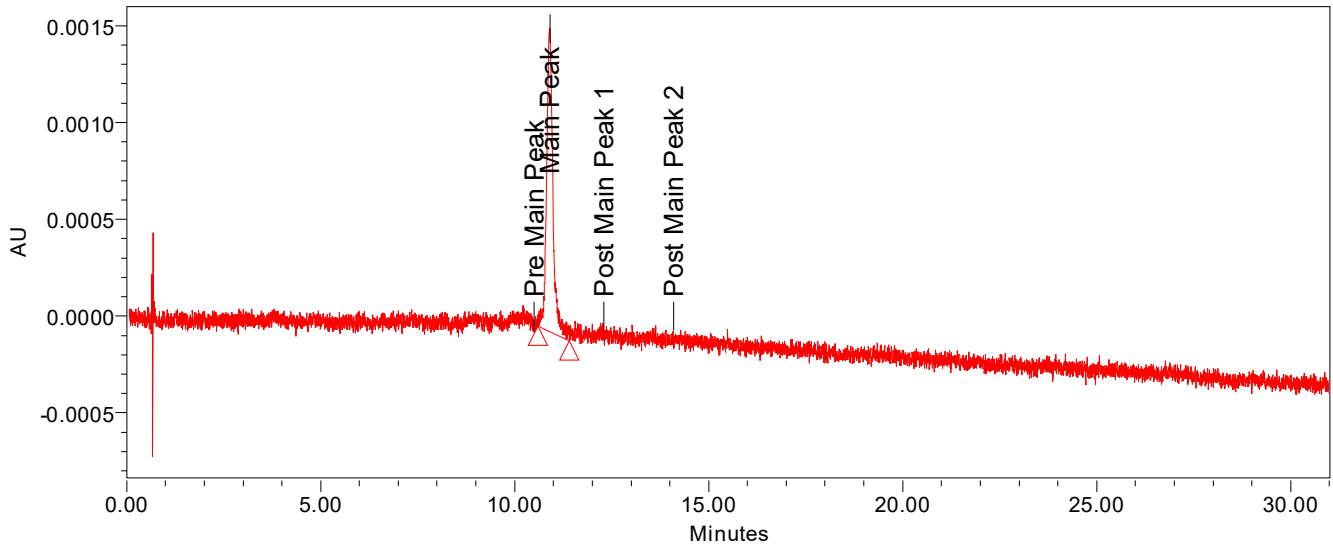
Component Results  
AreaRatio Summarized by Name

	SampleName	Label	IG: Lipid adducts	IG: RNA Fragments	Pre Main Peak	Main Peak
1	Water blank SST	B0				
2	Water blank	B2				0.1894
3	Water blank	B2				0.2053

Component Results  
AreaRatio Summarized by Name

1		
2		
3		

Sensitivity Solution



SampleName: Sensitivity; Vial: 1:E,3; Injection: 1; Channel Description PDA Ch1 260nm@4.8nm;  
 Channel PDA Ch1 260nm@4.8nm

Sensitivity Solution  
 s/n Summarized by Name

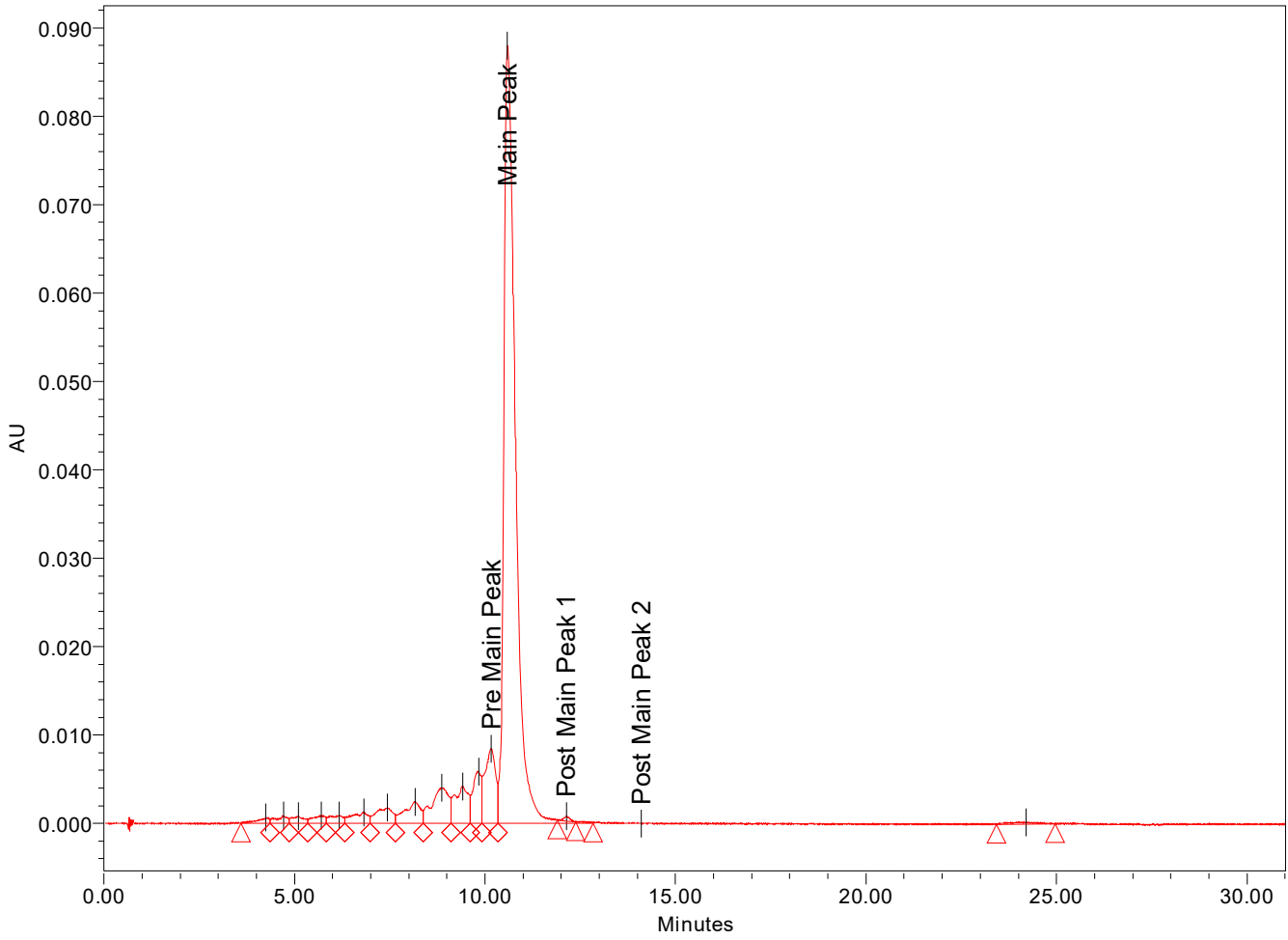
	IG: RNA Fragments	IG: Lipid adducts	Pre Main Peak	Main Peak	Post Main Peak 1
1				13.07	

Sensitivity Solution  
 s/n Summarized by  
 Name

	Post Main Peak 2
1	



Bracketing Reference Standard

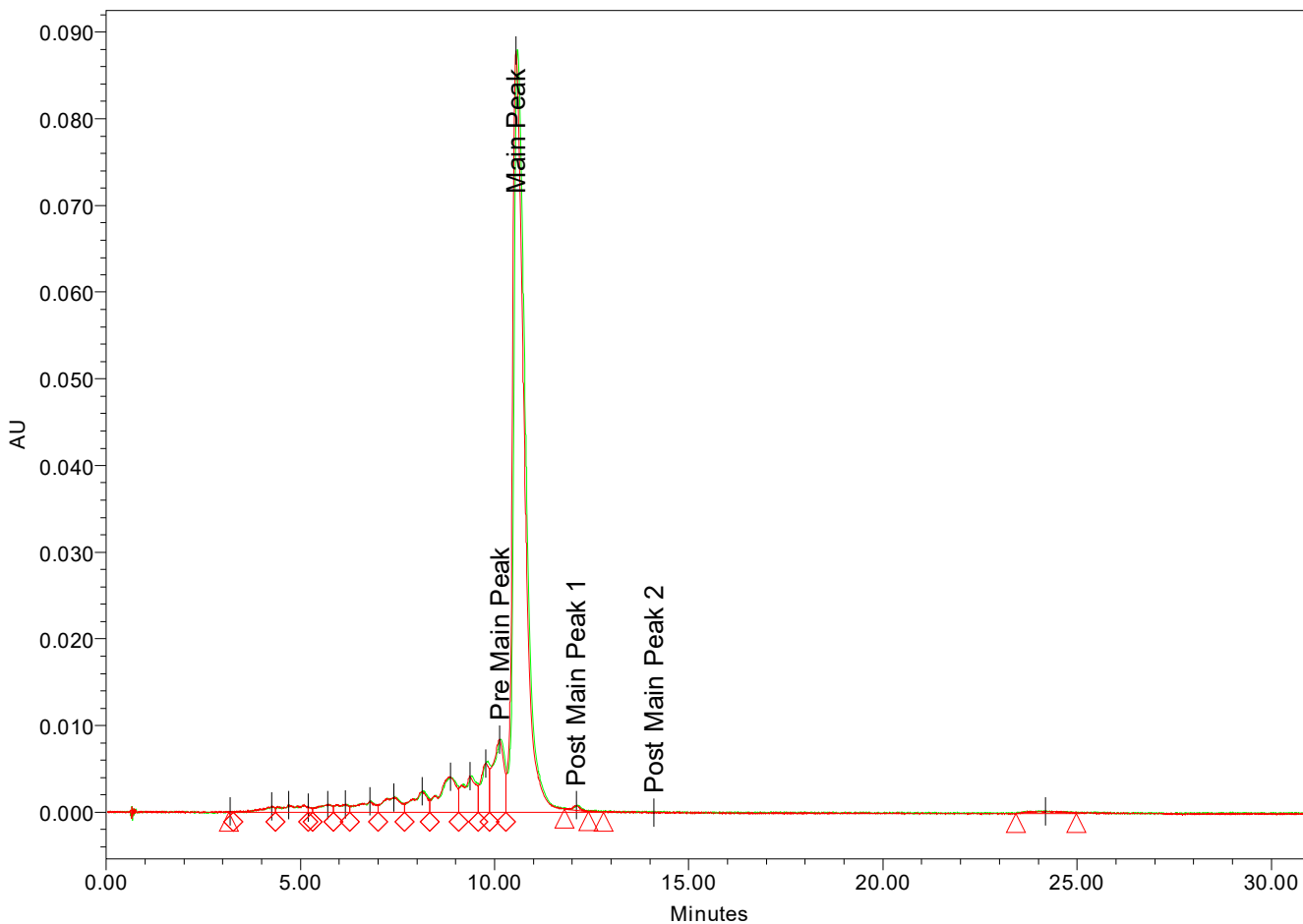


— SampleName: mRNA Std B; Vial: 1:A,4; Injection: 1; Label S0201

Component Summary Table  
Name: Main Peak

Sample Name	Name	Vial	Inj	Total Area	Label	Area (μV*sec)	Std_AreaRatio	Std_RT_ratio
1   mRNA Std B	Main Peak	1:A,4	1	2575464	S0201	1827799	100.1	100.39

Summary of Reference Standard Injections



— SampleName: mRNA Std A; Vial: 1:A,1; Injection: 1; Label S0101  
 — SampleName: mRNA Std B; Vial: 1:A,4; Injection: 1; Label S0201

Component Summary Table  
 Area Summarized by Name

	Sample Name	Inj	Total Area	Label	IG: RNA Fragments ( $\mu\text{V}\cdot\text{sec}$ )	Pre Main Peak ( $\mu\text{V}\cdot\text{sec}$ )	Main Peak ( $\mu\text{V}\cdot\text{sec}$ )	Post Main Peak 1 ( $\mu\text{V}\cdot\text{sec}$ )
1	mRNA Std A	1	2578283	S0101	730595	162730	1825886	6856
2	mRNA Std B	1	2575464	S0201	728432	164657	1827799	6422
Mean			2576874		729513.8	163693.3	1826842.5	6639.0
% RSD			0.1		0.2	0.8	0.1	4.6

Component Summary Table  
 Area Summarized by Name

	IG: Lipid adducts ( $\mu\text{V}\cdot\text{sec}$ )
1	21801
2	19233
Mean	20517.2
% RSD	8.9

Component Summary Table  
Std\_AreaRatio Summarized by Name

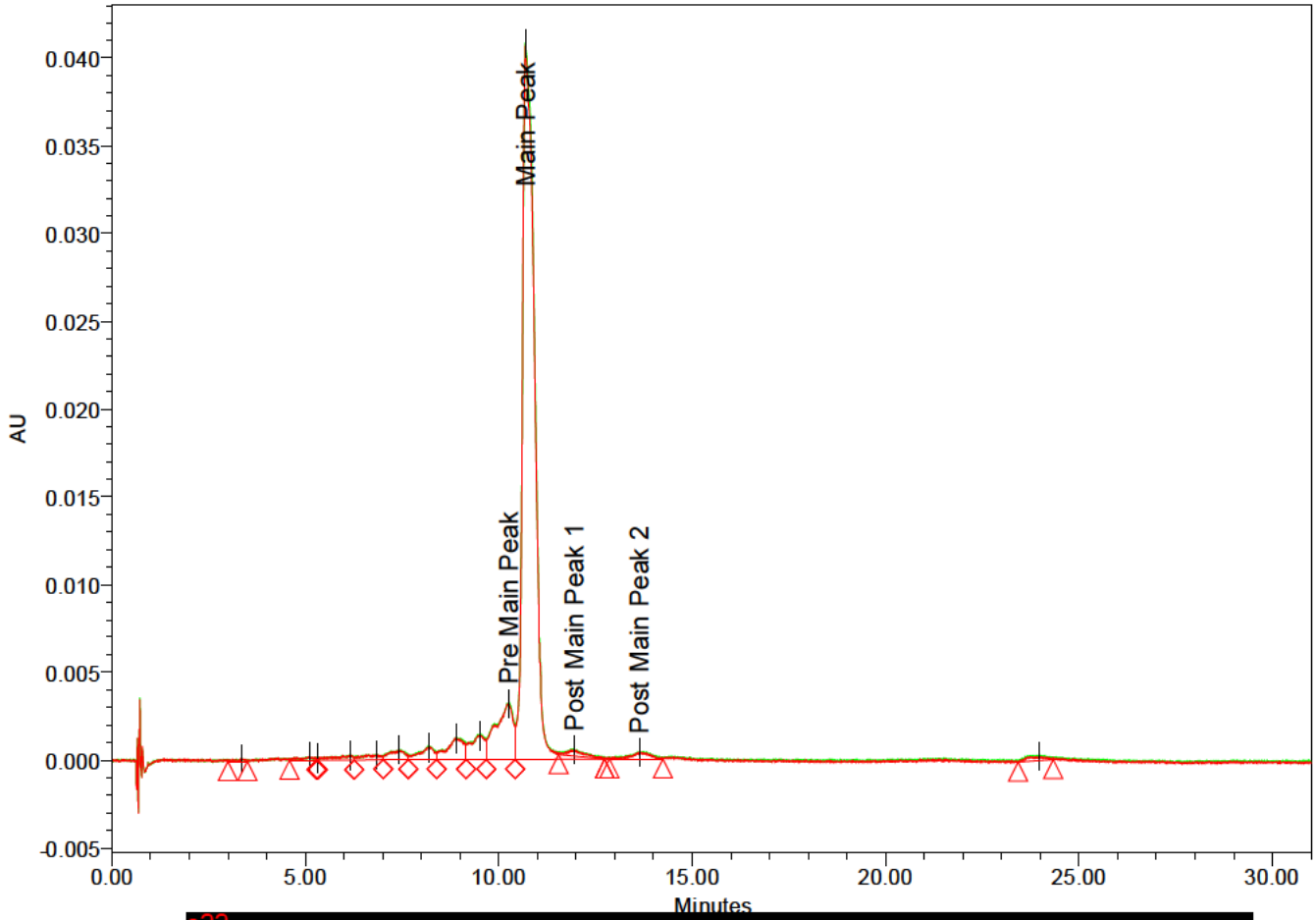
	Sample Name	Inj	Total Area	Label	IG: RNA Fragments	Pre Main Peak	Main Peak	Post Main Peak 1
1	mRNA Std A	1	2578283	S0101	100.0	100.0	100.0	100.0
2	mRNA Std B	1	2575464	S0201	99.7	101.2	100.1	93.7
Mean			2576874		99.9	100.6	100.1	96.8
% RSD			0.1		0.2	0.8	0.1	4.6

Component Summary Table  
Std\_AreaRatio Summarized  
by Name

	IG: Lipid adducts
1	100.0
2	88.2
Mean	94.1
% RSD	8.9

# Sample Results

Sample 1



s22 [Redacted]

% Area Summarized by Name  
Label: U0101

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts
1	s47	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]
2	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]
Mean	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]
% RSD	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]	[Redacted]

% Area Summarized by Name  
Label: U0101

1	s47	[Redacted]
2	[Redacted]	[Redacted]
Mean	[Redacted]	[Redacted]
% RSD	[Redacted]	[Redacted]

s22 [Redacted]

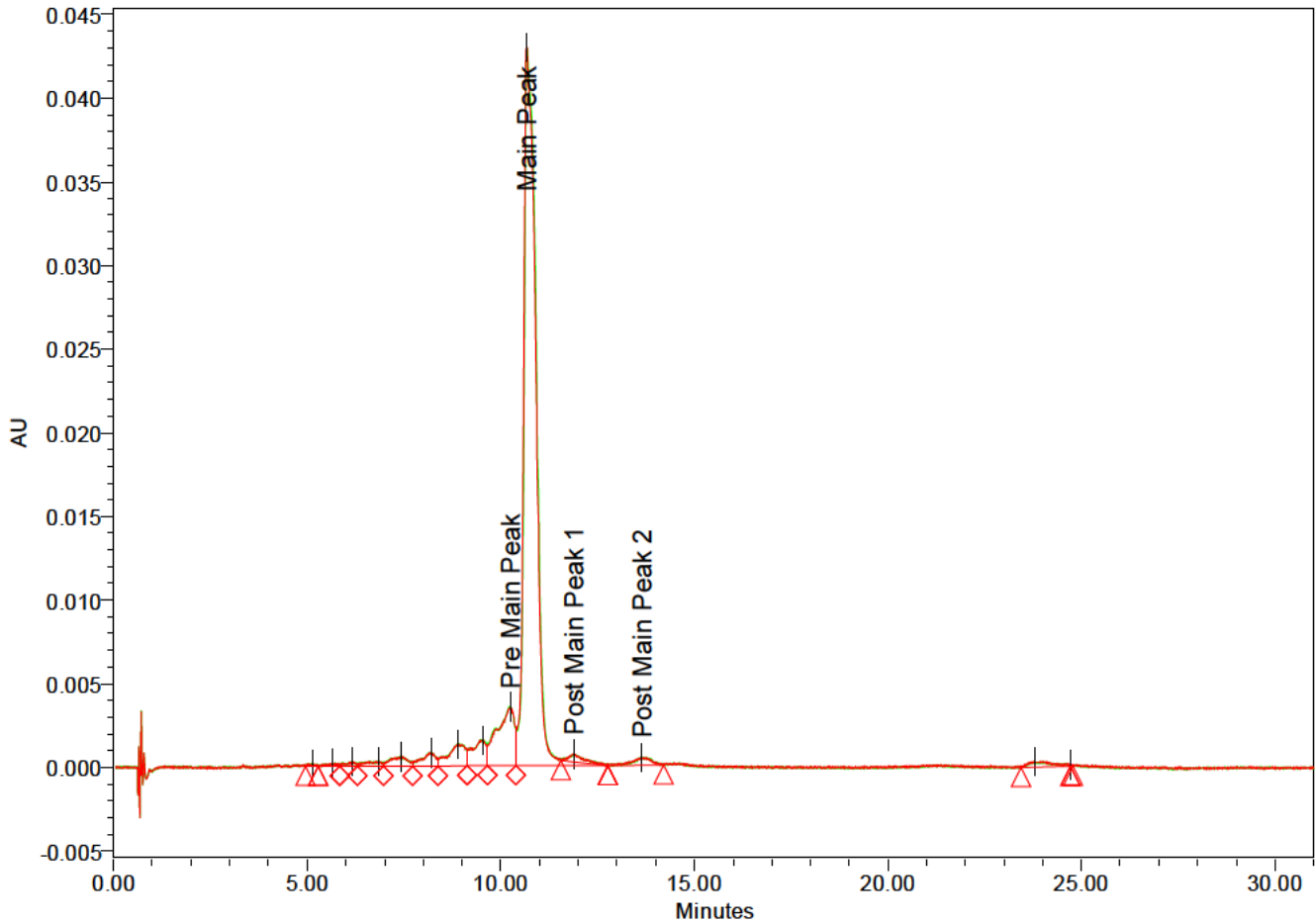
Sample\_AreaDif Summarized by Name  
Label: U0101

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts
1	s47						

Sample\_AreaDif Summarized by Name  
Label: U0101

1	s22						
---	-----	--	--	--	--	--	--

Sample 2



— SampleName: Spikevax Bivalent - 2208002750; Vial: 1:A,3; Injection: 1; Label U0102  
 — SampleName: Spikevax Bivalent - 2208002750; Vial: 1:A,3; Injection: 2; Label U0102

% Area Summarized by Name  
 Label: U0102

	Label	SampleName	Inj	Total Area	IG: RNA Fragments	Pre Main Peak	Main Peak
1	U0102	Spikevax Bivalent - 2208002750	1	s47			
2	U0102	Spikevax Bivalent - 2208002750	2				
Mean							
Std. Dev.							
% RSD							

% Area Summarized by Name  
 Label: U0102

	IG: Lipid adducts	Post Main Peak 1	Post Main Peak 2
1	s47		
2			
Mean			
Std. Dev.			
% RSD			

Sample\_AreaDif Summarized by Name  
Label: U0102

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts
1	U0102	Spikevax Bivalent - 2208002750	2	s47			

Sample\_AreaDif Summarized by Name  
Label: U0102

1	s47						
---	-----	--	--	--	--	--	--








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Error Log

Overlay Chromatogram group contains information that doesn't match the data being reported.  
Amount Component Summary group contains information that doesn't match the data being reported.  
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**Australian Government**  
**Department of Health**  
Therapeutic Goods Administration

Laboratories Branch

Operations: HPLC Manual	
Procedure	HPLC - 01 - General HPLC - WORKSHEET
Written	s22
Authorised	
Date issued	12/4/2019
Revision #	8

**HPLC - 01 - General HPLC - WORKSHEET**

TEST DETAILS			
TEST NAME	Analysis of mRNA purity in Moderna SpikeVax bivalent vaccine by Size-based RPIP		
METHOD REFERENCE	Moderna SOP-1142		
METHOD MODIFICATIONS (if any)	None		
MODIFICATIONS APPROVED BY:	N/A		
NAME OF ANALYST	s22	TEST DATE	31-Aug-2022

BUFFERS AND SOLUTIONS	
SOLUTIONS	BATCH No:
MOBILE PHASE A	ST29Aug22-1
MOBILE PHASE B	ST29Aug22-2
SAMPLE DILUENT	

INTERNAL USE ONLY

IN CONFIDENCE

**PIPETTES USED AND EXPIRY DATES**

LIMS# 32837, Exp: 21/10/22  
 LIMS#33250, Exp: 07/10/22  
 LIMS#32677, Exp: 06/10/22

**REFERENCE MATERIALS**

NAME AND CODE	BATCH NO:
mRNA Ref. Std, CX-024414, Conc: 3.2mg/ml	RM-4007421130

**REFERENCE MATERIAL PREPARATIONS AND CALCULATIONS**

Conc. of Ref. Std 3.2mg/ml  
 Required/Working concentration: 0.2mg/ml  
 Stock Ref was diluted to 16x with RNase free water  
 100ul of stock Ref. Std. was mixed with 1500ul of DEPC water to make 0.2mg/ml

INTERNAL USE ONLY

IN CONFIDENCE

SYSTEM SUITABILITY CRITERIA AND RESULTS			
PARAMETERS	LIMITS	RESULTS	COMMENTS
No peak in the water blank injection prior to the Sensitivity Solution	No peak	Conforms	Pass
All other carryover water blank injections in the sequence interfere with the peaks of interest as compared to the total peak area of the mRNA working reference standard	Interference $\leq 1\%$	Conforms	Pass
Signal to Noise ratio of Sensitivity Solution	$\geq 10$	13.07	Pass
% Recovery of the main peak area in each bracketing standard compared to the peak area of the mRNA working reference standard	90-110%	100.1	Pass
% Agreement of the main peak RT in each bracketing standard compared to the main peak RT of the mRNA working reference standard	90-110%	100.39	Pass
Absolute Difference of the Main Peak % Area for duplicate sample preparations - 2208002748	$\leq 5\%$	2.43	Pass
Absolute Difference of the Main Peak % Area for duplicate sample preparations - 2208002750	$\leq 5\%$	1.481	Pass

S22



INTERNAL USE ONLY

IN CONFIDENCE

SAMPLE DETAILS			
SAMPLE NAME	Spikevax Bivalent elasomera & imelasomera 0.1 mg/ml sus. for inj. vial		
LIMS No:	2208002750-R1		
BATCH No:	000462A	EXPIRY:	23-Apr-2023

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
0.1mg/ml	-	-	0.1mg/ml	-	10ul
No dilution required. 100ul of neat sample taken into UPLC total recovery vial for injection.					
DATA LOCATIONS					
Copies of Empower reports attached?	Yes	Data location in TRIM	E22-542283		

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS
IG: Fragments	s47		
IG: Lipid adducts			
Main peak			

SAMPLE RESULTS: Pass

**INTERNAL USE ONLY**

**IN CONFIDENCE**

SAMPLE DETAILS			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

SAMPLE DILUTIONS, CALCULATIONS and DATA					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

**DATA LOCATIONS**

Copies of Empower reports attached?		Data location in TRIM	
-------------------------------------	--	-----------------------	--

TEST RESULTS			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:



**INTERNAL USE ONLY**

**IN CONFIDENCE**

<b>SAMPLE DETAILS</b>			
SAMPLE NAME			
LIMS No:			
BATCH No:		EXPIRY:	

<b>SAMPLE DILUTIONS, CALCULATIONS and DATA</b>					
Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.

<b>DATA LOCATIONS</b>		
Copies of Empower reports attached?	Data location in TRIM	

<b>TEST RESULTS</b>			
PARAMETERS	LIMITS	RESULTS	FIDUCIAL LIMITS

SAMPLE RESULTS:

**Comments**



Australian Government

Department of Health and Ageing  
Therapeutic Goods Administration

## Sample Set Summary Report

Sample Set: Sys 3\_Bivalent\_NewRPIP\_31Aug22

## Sample Set Information

Project Name: Biochemistry\2022\Spikevax RPIP

Sample Set Name: Sys  
3\_Bivalent\_NewRPIP\_31Aug22

Sample Set Acquired By: s22

Start Date: 31/08/2022 12:40:56 PM AEST

Finish Date: 31/08/2022 11:56:57 PM AEST

Run Time: 31.00 Minutes

Sample Set Altered: No

SampleName: mRNA Std B, Spikevax Bivalent -  
2208002750, Conditioning , Conditioning H2O, Water  
blank SST, Water blank, Spikevax Bivalent -  
2208002748, Sensitivity, mRNA Std A, Water blank

Acq Method Set: Spikevax RPIP\_2022\_MS

Instrument Method Name: Spikevax RPIP\_2022\_IM

Sample Set Method: !QuickSet

## System Information

System Name: System 3

Empower Node: Ucdpjpncwck2

Analytical\_Column\_1: ID#518 ProSwift RP-1S 4.6x50mm  
Sn002703

Analytical\_Column\_2:

## Processing Information

Processing Method: Spikevax RPIP 2022\_v3

Processed By: s22/Biochem

Processing Method Id: 5540

Date Processed: 13/09/2022 11:37:50 AM AEST,  
13/09/2022 11:37:53 AM AEST, 13/09/2022 11:37:56

Processing Node: Uclpbh7In13

Result Set Name: Sys

3\_Bivalent\_NewRPIP\_31Aug22

Result Set Date: 13/09/2022 11:37:46 AM AEST

Result Set Id: 5555

Channel Description: PDA Ch1 260nm@4.8nm

## Reporting Information

Report Method Name: Sample Set Summary Report

Print Date: 13/09/2022

Reported by: s22

Time: 11:40:14 AM Australia/ACT

## Injection Sequence Summary

	SampleName	Sample Type	Vial	Inj #	Run Time (Minutes)	Injection Volume (ul)	Sample Weight	Dilution	Level	Label
1	Conditioning	Control	1:E,1	1	31.00	10.00	1.00000	1.00000		
2	Conditioning	Control	1:E,1	2	31.00	10.00	1.00000	1.00000		
3	Conditioning	Control	1:E,1	3	31.00	10.00	1.00000	1.00000		
4	Conditioning	Control	1:E,1	4	31.00	10.00	1.00000	1.00000		
5	Conditioning	Control	1:E,1	5	31.00	10.00	1.00000	1.00000		
6	Conditioning H2O	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
7	Conditioning H2O	Control	1:E,2	2	31.00	10.00	1.00000	1.00000		
8	Conditioning H2O	Control	1:E,2	3	31.00	10.00	1.00000	1.00000		
9	Conditioning H2O	Control	1:E,2	4	31.00	10.00	1.00000	1.00000		
10	Water blank SST	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B0
11	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B1
12	Sensitivity	Control	1:E,3	1	31.00	10.00	1.00000	1.00000		A
13	mRNA Std A	Standard	1:A,1	1	31.00	10.00	1.00000	1.00000		S0101
14	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B2
15	Spikevax Bivalent - 2208002748	Unknown	1:A,2	1	31.00	10.00	1.00000	1.00000		U0101
16	Spikevax Bivalent - 2208002748	Unknown	1:A,2	2	31.00	10.00	1.00000	1.00000		U0101
17	Spikevax Bivalent - 2208002750	Unknown	1:A,3	1	31.00	10.00	1.00000	1.00000		U0102
18	Spikevax Bivalent - 2208002750	Unknown	1:A,3	2	31.00	10.00	1.00000	1.00000		U0102
19	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
20	mRNA Std B	Standard	1:A,4	1	31.00	10.00	1.00000	1.00000		S0201
21	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B2



<b>Type:</b> Biotherapeutics\BEE\Forms	<b>Number:</b> Bio-BEE-Form-39 / <b>Version:</b> 3
<b>Owner:</b> s22	<b>Approver:</b> s22
<b>Active:</b> 10/05/2022	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Endotoxin Routine Assay Worksheet	

## Endotoxin Routine Assay Worksheet

Assay ID: 26Aug2022 Operator: s22

### Limulus Amoebocyte Lysate (LAL)

Lysate batch and expiry recorded on software for each assay

Ensure sensitivity of LAL batch has been confirmed. 'Lysate preparation details' shown below

### Recombinant Factor C (rFC)

rFC Enzyme, Fluorogenic Substrate & rFC Assay Buffer batches and expiry dates recorded on software for each assay

Ensure sensitivity of the rFC batch has been confirmed. 'rFC Reagent preparation details' shown below

### Control Standard Endotoxin (CSE) – refer to Bio-BEE-Method 5 and Bio-BEE-Form 37

CSE batch and expiry recorded on software for each assay

Reconstitution details for either KLAL or rFC – see Trim File D22-5818475

CSE Lot Number: 0000981165 Conc 50 EU/mL

### LAL Reagent Water (LRW) Lot Number: 0000966190 Expiry: 20Jul2023

How many samples were linked to this assay? 4

**This form is used for recording the assay details and results and only gives the method in point form. See Bio-BEE-SOP 28 and appropriate method for the detailed procedure.**

To avoid endotoxin contamination, use careful technique and **pyrogen free** equipment.

### Preparation of Assay

- Fill out the appropriate forms from the Quality Management System (QMS)
- Retrieve the required kit reagents from cold storage to equilibrate to room temperature
- Turn on plate reader and computer and follow the steps as detailed in Bio-BEE-Method 4

### Preparation of CSE (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare CSE as detailed in Bio-BEE-Method 5. CSE dilutions can be dispensed to the plate as they are prepared to save mixing time

- CSE is set up as in the table below - record %CV results from the final report

#### For a KLAL (KQCL) assay

Concentration	Plate wells ID	% CV
50 EU/ml	F1 – F2	0.35
5 EU/ml	E1 – E2	0.39
0.5 EU/ml	D1 – D2	0.98
0.05 EU/ml	C1 – C2	0.80
0.005 EU/ml	B1 – B2	0.81
Blank	A1 – A2	

#### For an rFC assay

Concentration	Plate wells ID	% CV
5 EU/ml	E1 – E2	N/A
0.5 EU/ml	D1 – D2	N/A
0.05 EU/ml	C1 – C2	N/A
0.005 EU/ml	B1 – B2	N/A
Blank	A1 – A2	

- Dispense 100 µl of the appropriate dilution of CSE into the appropriate wells of the plate.
- Continue with procedure as per the appropriate method

### Preparation of Samples (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare sample dilutions as in Bio-BEE-Form 42
- Pipette 10 µl of the 5 EU/ml standard to the appropriate PPC wells as per the plate layout
- Dispense 100 µl of the final sample dilutions into the 4 appropriate wells as per the plate layout

### Starting the Assay

- The plate is then ready for the reaction. Prepare the software as set out in the Bio-BEE-Method 4
- “Run” the Template prepared earlier. Follow the prompts to the Pre-warming step

#### If performing a KLAL assay

- Prepare the required lysate vial/s (Bop-BEE-Method 6) and pour into the reagent reservoir

Lysate Preparations Details -

Lysate Lot Number	XL021QDD4P	Expiry 17Aug2023
Date sensitivity confirmed	09May2022	TRIM#: E22-564008
Reconstitute lysate with	2.6mL (2 x 2mL + 600uL)	mL of LAL Reagent Water (LRW)
Date reconstituted	26Aug2022	Click or tap here to enter text.
Operator(s)	s22	Click or tap here to enter text.
Use by date	09Sep2022	(Lonza KQCL- 14 days from reconstitution, at below -10°C)

**OR**

*If performing an rFC assay*

- Make up the required volume of rFC reagent directly into the reservoir (Bio-BEE-Method 6)

rFC Reagent Preparations Details – (Note: once prepared, working reagent cannot be stored)

	Lot	Expiry	Volume
Date sensitivity confirmed	N/A	N/A	
Fluorogenic Substrate	N/A	N/A	N/A µL
rFC Assay Buffer	N/A	N/A	N/A µL
rFC Enzyme Solution	N/A	N/A	N/A µL

- Open cover – if using the Spectramax use the software to open and close the drawer
- Add 100 µl of either lysate (KLAL) **OR** working reagent (rFC) to each of the assay wells, carefully, and as quickly as possible
- Close the drawer on the plate reader and click OK to start the run. **Do not open drawer**

**Acceptance Criteria – for KLAL**

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>-0.997</u>
Slope between -0.400 and -0.100	<u>-0.214</u>
Y intercept between 2.500 and 3.500	<u>3.125</u>
Mean reaction times of blank $\geq$ mean reaction times of lowest standard	<u>Yes</u>
Coefficient of variation (CV) values for all standards are $< 10\%$	<u>Yes</u>
Were all acceptance criteria for the standard curve met?	<u>Yes</u>

## Acceptance Criteria – for rFC

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>N/A</u>
Slope between 0.760 and 1.110	<u>N/A</u>
Y intercept between 2.500 and 5.000	<u>N/A</u>
Mean RFU of blank $\leq$ mean RFU of lowest standard	<u>N/A</u>
Coefficient of variation (CV) values for all standards are $< 25\%$	<u>N/A</u>
Were all acceptance criteria for the standard curve met?	<u>N/A</u>

## Conclusions

Follow procedures for 'Recording Results' detailed in Bio-BEE-Method 7

## Notes

The water Batch number in the Endotoxin report was entered wrong, not 0001050844 it should be 0000966190 (Exp 20/07/2023). s22 26Aug2022

Checked s22 26Aug2022





Australian Government  
Department of Health and Ageing  
Therapeutic Goods Administration

2303001127, 2303001129, mRNA Std B, Conditioning ,  
Conditioning H2O, Water blank SST, Water blank,  
Sensitivity. 2303001131. mRNA Std A. Water blank

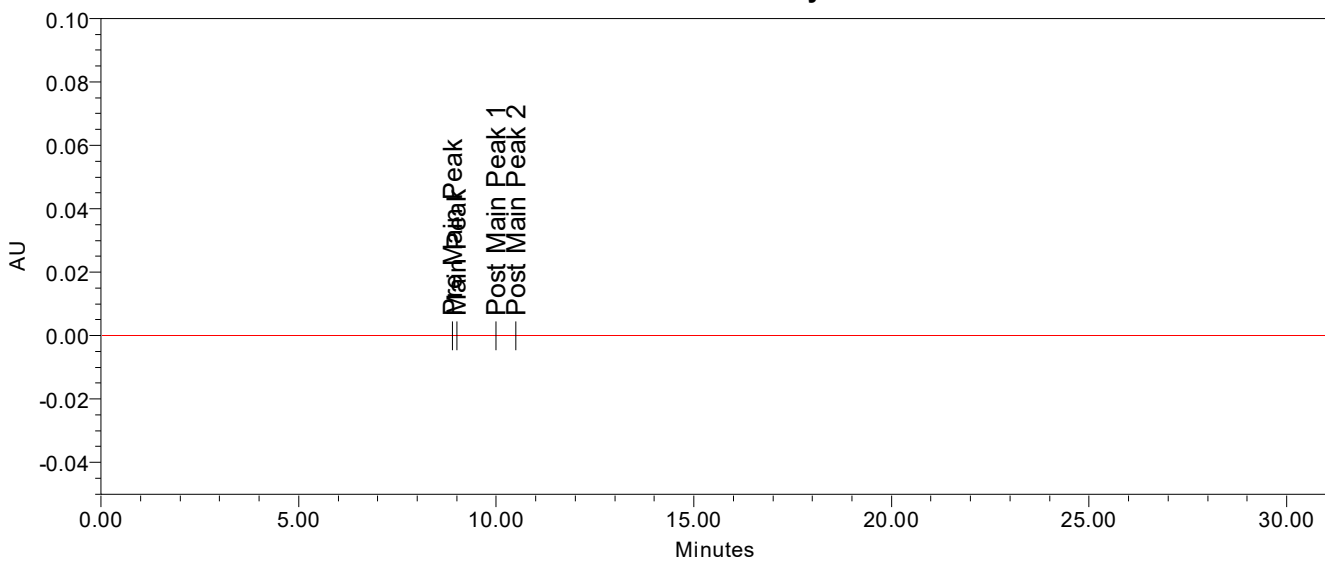
Sample Set Name: Spikevax 23030001127

08Mar23

Sample Set Acquired By: s22

## System Suitability

Water blank - Prior to sensitivity standard

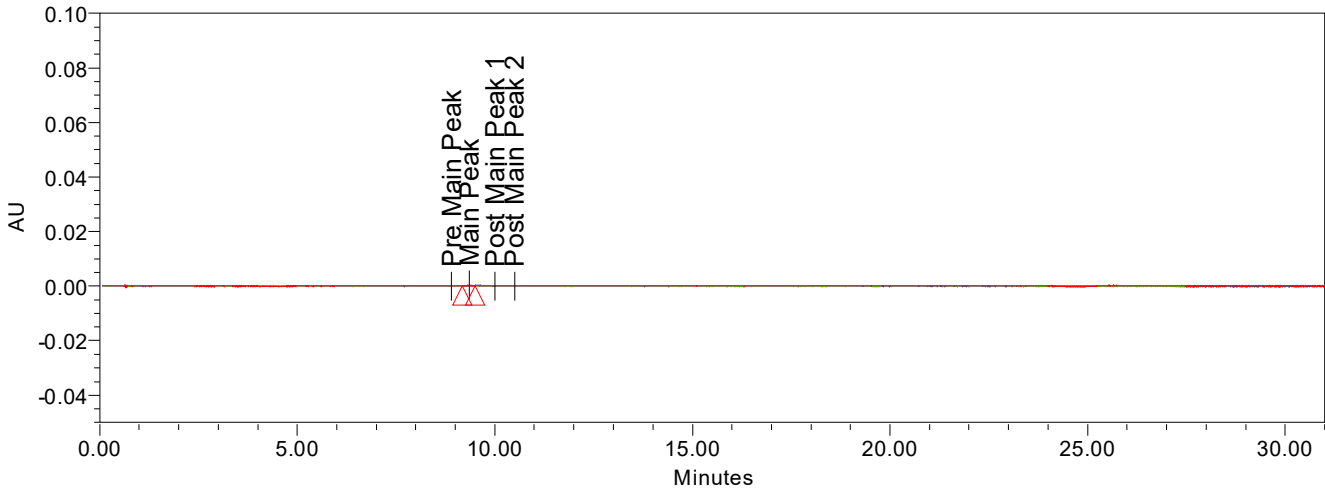


— Label B1; SampleName: Water blank; Vial: 1:E,2; Injection: 1

Component Results

	SampleName	Name	RT	Height	% Area	AreaRatio	Area (μV*sec)
1	Water blank	IG: RNA Fragments					
2	Water blank	IG: Lipid adducts					
3	Water blank	Pre Main Peak	8.900				
4	Water blank	Main Peak	9.000				
5	Water blank	Post Main Peak 1	10.000				
6	Water blank	Post Main Peak 2	10.500				

Water blank - Carryover assessment



- Label B2; SampleName: Water blank; Vial: 1:E,2; Injection: 1
- Label B0; SampleName: Water blank SST; Vial: 1:E,2; Injection: 1
- Label B2; SampleName: Water blank; Vial: 1:E,2; Injection: 1

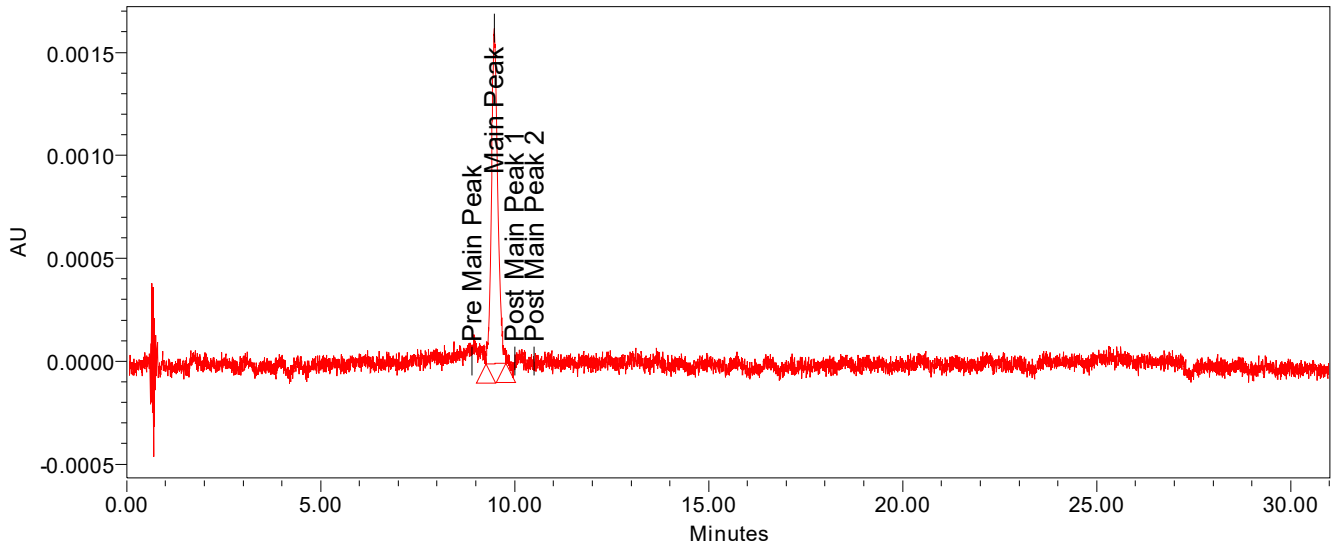
Component Results  
AreaRatio Summarized by Name

	SampleName	Label	IG: RNA Fragments	IG: Lipid adducts	Pre Main Peak	Main Peak
1	Water blank SST	B0				
2	Water blank	B2				0.1130
3	Water blank	B2				0.1592

Component Results  
AreaRatio Summarized by Name

	Post Main Peak 1	Post Main Peak 2
1		
2		
3		

Sensitivity Solution



SampleName: Sensitivity; Vial: 1:E,3; Injection: 1; Channel Description PDA Ch1 260nm@4.8nm;  
Channel PDA Ch1 260nm@4.8nm

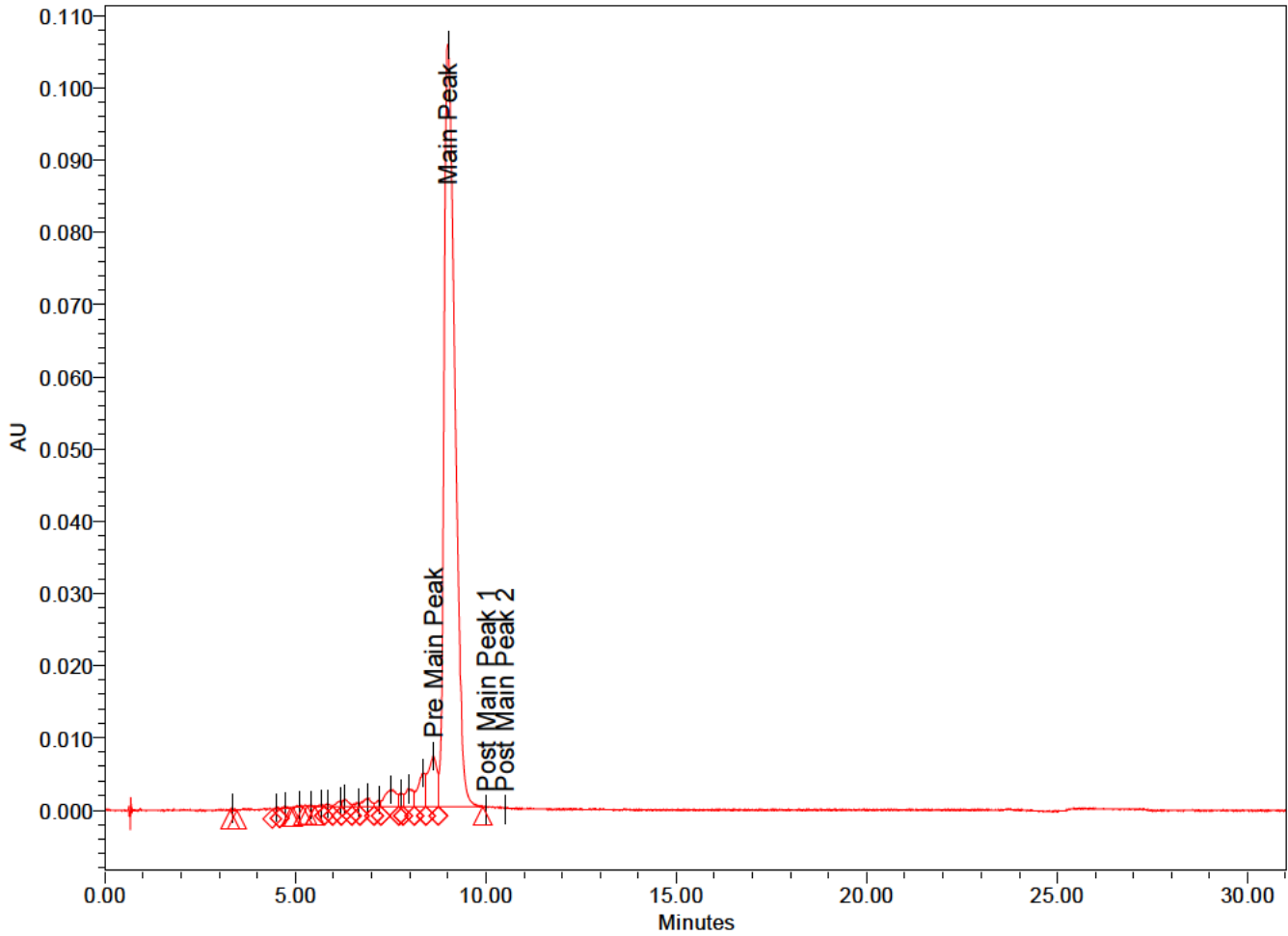
Sensitivity Solution  
s/n Summarized by Name

	IG: RNA Fragments	IG: Lipid adducts	Pre Main Peak	Main Peak	Post Main Peak 1
1				s47	

Sensitivity Solution  
s/n Summarized by  
Name

	Post Main Peak 2
1	

Bracketing Reference Standard

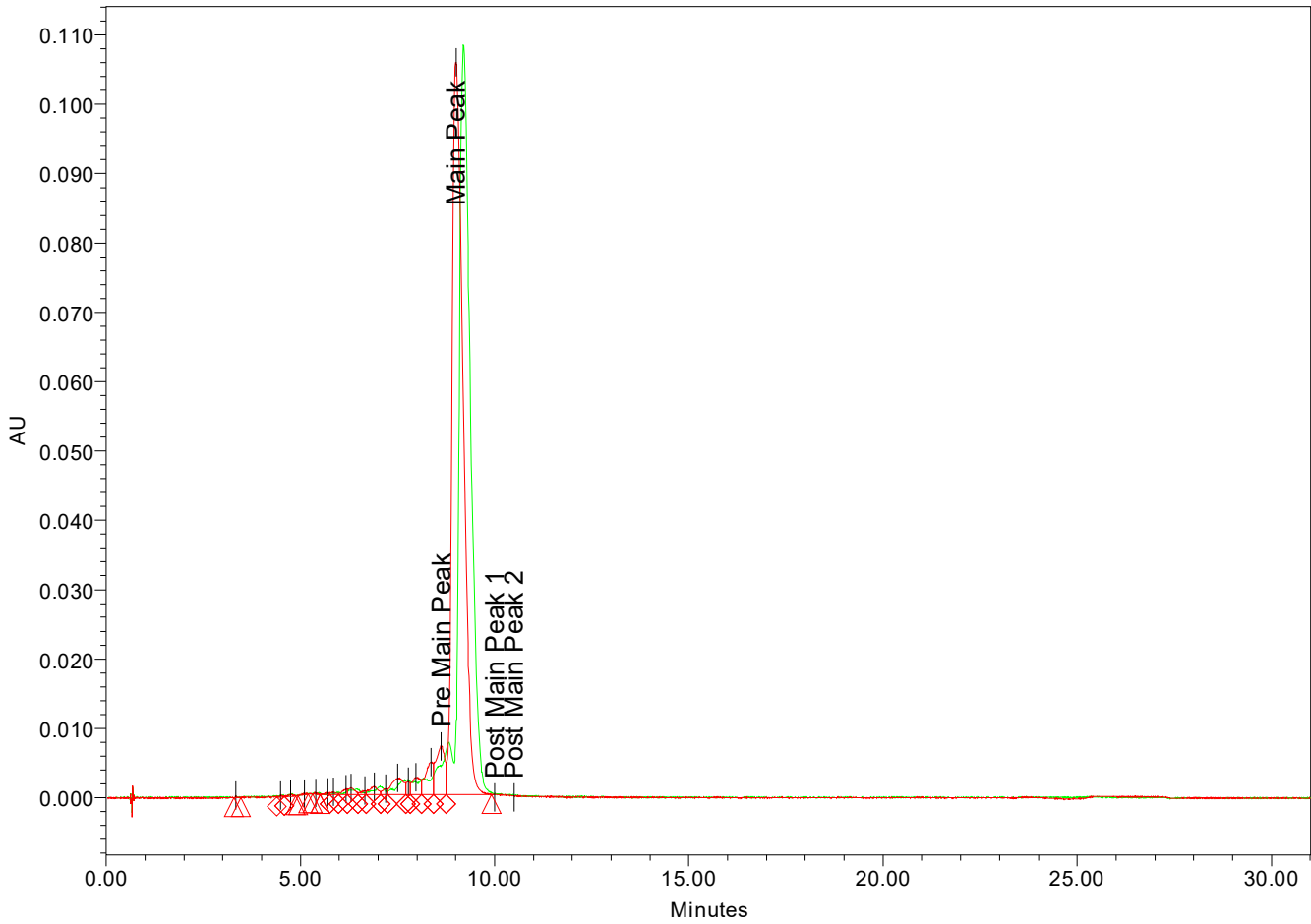


— SampleName: mRNA Std B; Vial: 1:A,8; Injection: 1; Label S0201

Component Summary Table  
Name: Main Peak

Sample Name	Name	Vial	Inj	Total Area	Label	Area (µV*sec)
1 mRNA Std B	Main Peak	1:A,8	1	2452722	S0201	2112465

Summary of Reference Standard Injections



— SampleName: mRNA Std B; Vial: 1:A,8; Injection: 1; Label S0201  
 — SampleName: mRNA Std A; Vial: 1:A,1; Injection: 1; Label S0101

Component Summary Table  
 Area Summarized by Name

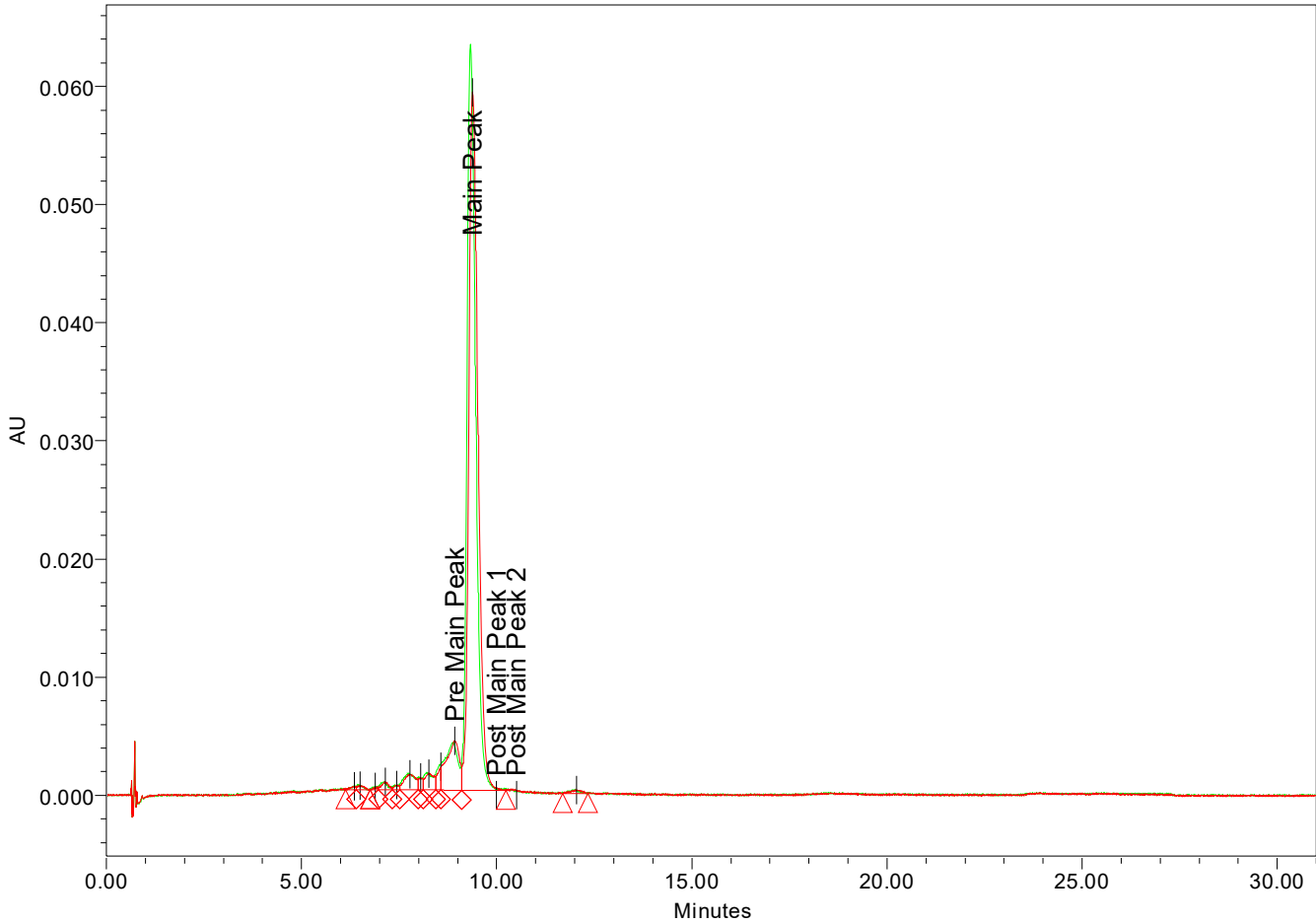
	Sample Name	Inj	Total Area	Label	IG: RNA Fragments ( $\mu\text{V}\cdot\text{sec}$ )	Pre Main Peak ( $\mu\text{V}\cdot\text{sec}$ )	Main Peak ( $\mu\text{V}\cdot\text{sec}$ )
1	mRNA Std A	1	2458286	S0101	357681	136951	2100604
2	mRNA Std B	1	2452722	S0201	340256	111809	2112465
Mean			2455504		348968.9	124380.1	2106534.8
% RSD			0.2		3.5	14.3	0.4

Component Summary Table  
 Std\_AreaRatio Summarized by Name

	Sample Name	Inj	Total Area	Label	IG: RNA Fragments	Pre Main Peak	Main Peak
1	mRNA Std A	1	2458286	S0101	100.0	100.0	100.0
2	mRNA Std B	1	2452722	S0201	95.1	81.6	100.6
Mean			2455504		97.6	90.8	100.3
% RSD			0.2		3.5	14.3	0.4

# Sample Results

Sample 1



— SampleName: 2303001127; Vial: 1:A,2; Injection: 1; Label U0101  
 — SampleName: 2303001127; Vial: 1:A,3; Injection: 1; Label U0101

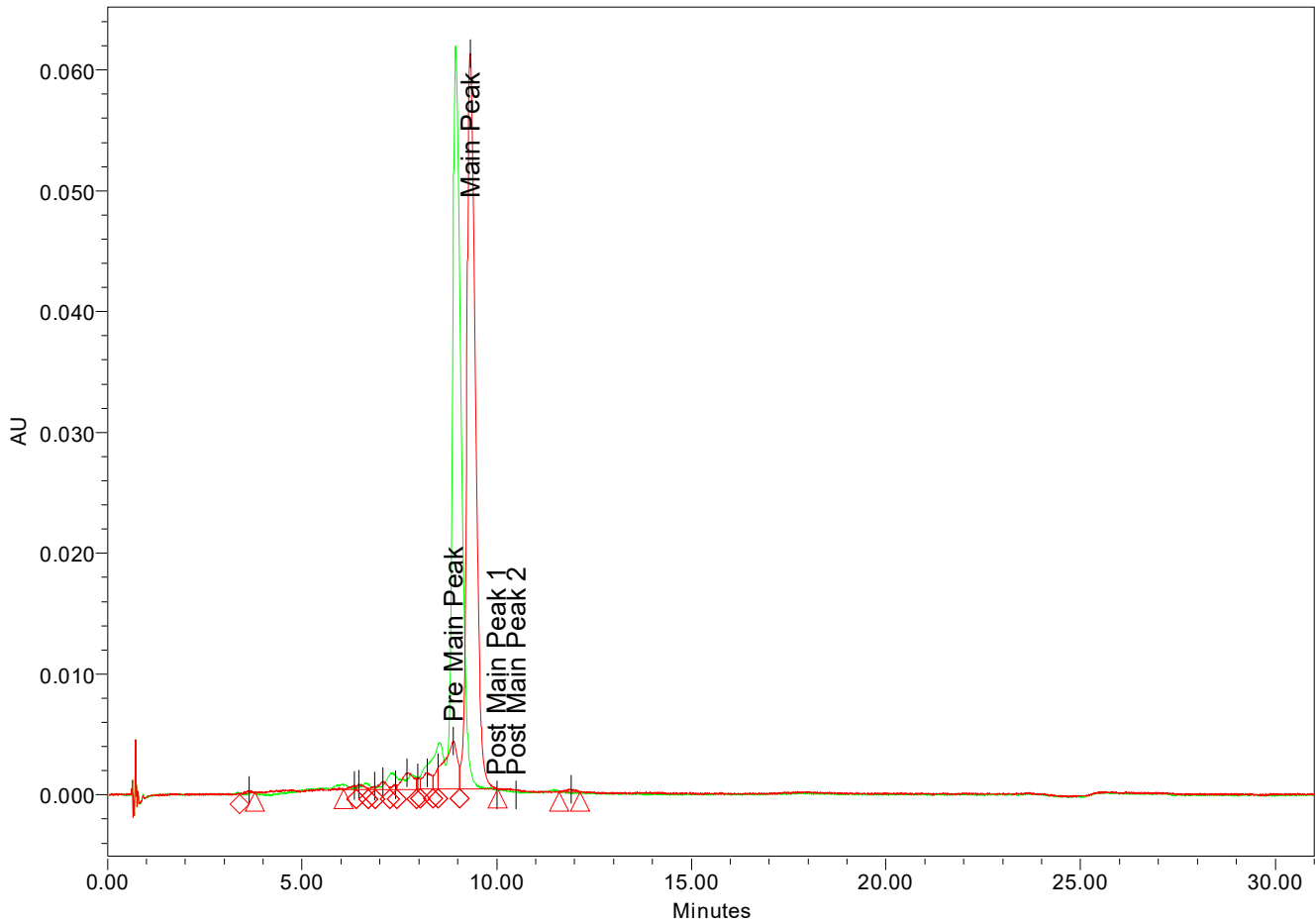
% Area Summarized by Name  
 Label: U0101

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts
1	U0101	2303001127	1	16.042	8.179	83.511	0.447
2	U0101	2303001127	1	16.079	8.012	83.494	0.427
Mean				S47	8.1	S47	S47
% RSD					1.5		

Sample\_AreaDif Summarized by Name  
 Label: U0101

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts
1	U0101	2303001127	1	16.042	8.179	83.511	0.447
2	U0101	2303001127	1	16.079	8.012	83.494	0.427

Sample 2



— SampleName: 2303001129; Vial: 1:A,4; Injection: 1; Label U0102  
 — SampleName: 2303001129; Vial: 1:A,5; Injection: 1; Label U0102

% Area Summarized by Name  
 Label: U0102

	Label	SampleName	Inj	Total Area	IG: RNA Fragments	Pre Main Peak	Main Peak	Post Main Peak 1
1	U0102	2303001129	1	1134143	16.1	8.1	83.5	
2	U0102	2303001129	1	1107433	14.4	7.7	85.4	
Mean				1120788	s47	7.9	s47	
Std. Dev.				18887	1.2	0.2	1.3	
% RSD				1.7	s47	2.9	s47	

% Area Summarized by Name  
 Label: U0102

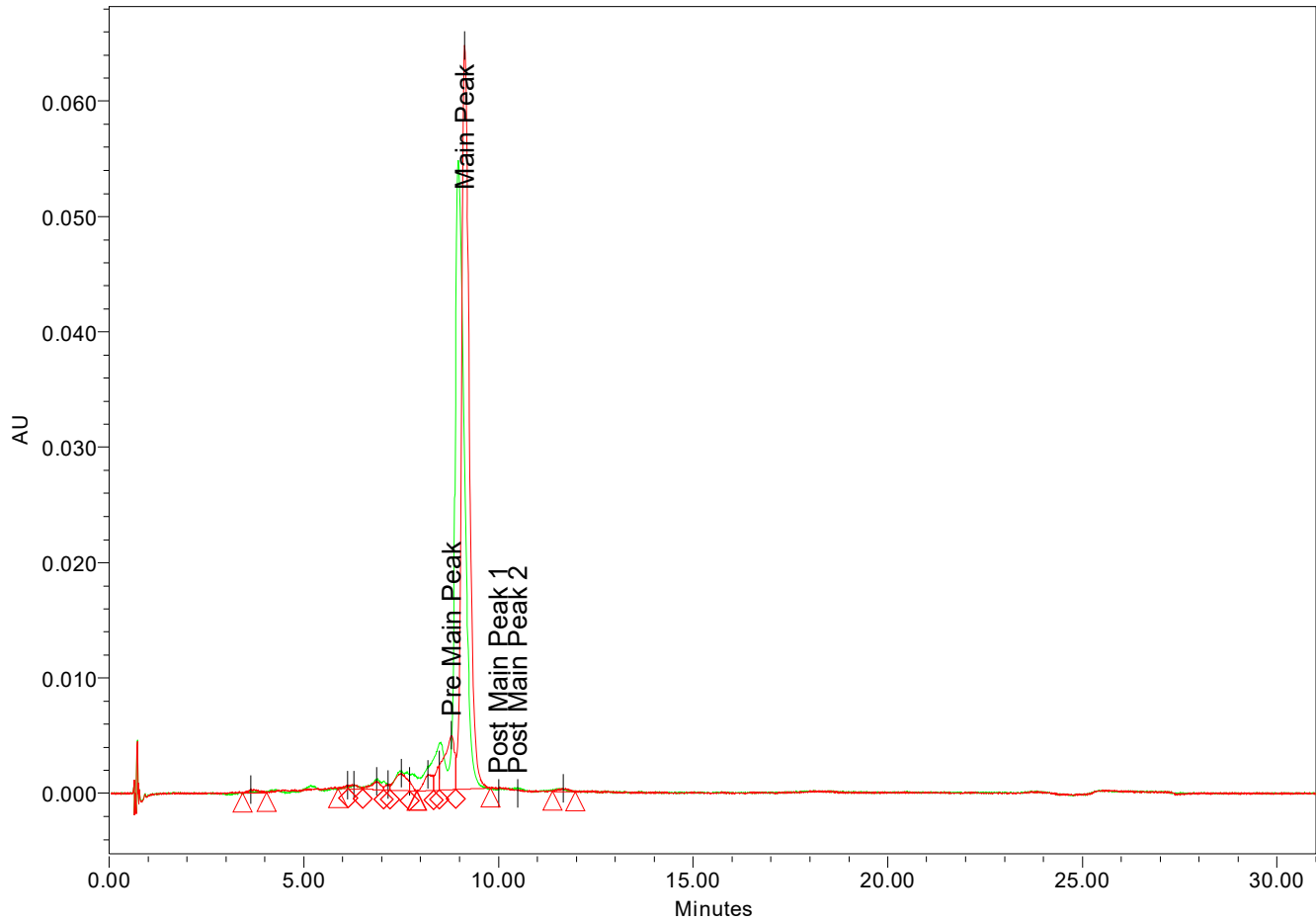
	Post Main Peak 2	IG: Lipid adducts
1		0.4
2		0.2
Mean		s47
Std. Dev.		0.1
% RSD		s47

Sample\_AreaDif Summarized by Name  
Label: U0102

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts
1	U0102	2303001129	1	16.115	8.075	S47	0.361
2	U0102	2303001129	1	14.385	7.746		0.240



Sample 3



— SampleName: 2303001131; Vial: 1:A,7; Injection: 1; Label U0103  
 — SampleName: 2303001131; Vial: 1:A,6; Injection: 1; Label U0103

% Area Summarized by Name  
 Label: U0103

	Label	SampleName	Inj	Total Area	IG: RNA Fragments	Pre Main Peak	Main Peak	Post Main Peak 1
1	U0103	2303001131	1	1088016	16.7	8.2	82.6	
2	U0103	2303001131	1	1081035	17.5	8.2	82.1	
Mean				1084525	s47	8.2	s47	
Std. Dev.				4936	0.5	0.0	0.4	
% RSD				0.5	s47	0.2	s47	

% Area Summarized by Name  
 Label: U0103

	IG: Lipid adducts	Post Main Peak 2
1	0.6	0.3
2	0.5	
Mean	s47	0.3
Std. Dev.	0.1	
% RSD	s47	

Sample\_AreaDif Summarized by Name  
Label: U0103

	Label	SampleName	Inj	IG: RNA Fragments	Pre Main Peak	Main Peak	IG: Lipid adducts	Post Main Peak 2
1	U0103	2303001131	1	16.735	8.186	S47	0.635	0.259
2	U0103	2303001131	1	17.461	8.166		0.471	



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Error Log

Overlay Chromatogram group contains information that doesn't match the data being reported.

Amount Component Summary group contains information that doesn't match the data being reported.

Amount Component Summary group contains information that doesn't match the data being reported.

Overlay Chromatogram group contains information that doesn't match the data being reported.

Amount Component Summary group contains information that doesn't match the data being reported.

Amount Component Summary group contains information that doesn't match the data being reported.



<b>Type:</b> Biotherapeutics\BPC\Forms	<b>Number:</b> Bio-BPC-Form-10 / <b>Version:</b> 2
<b>Owner:</b> s22	<b>Approver:</b> s22
<b>Active:</b> 14/11/2022	<b>Review:</b> 20/07/2022
<b>Title:</b> HPLC – General Worksheet	

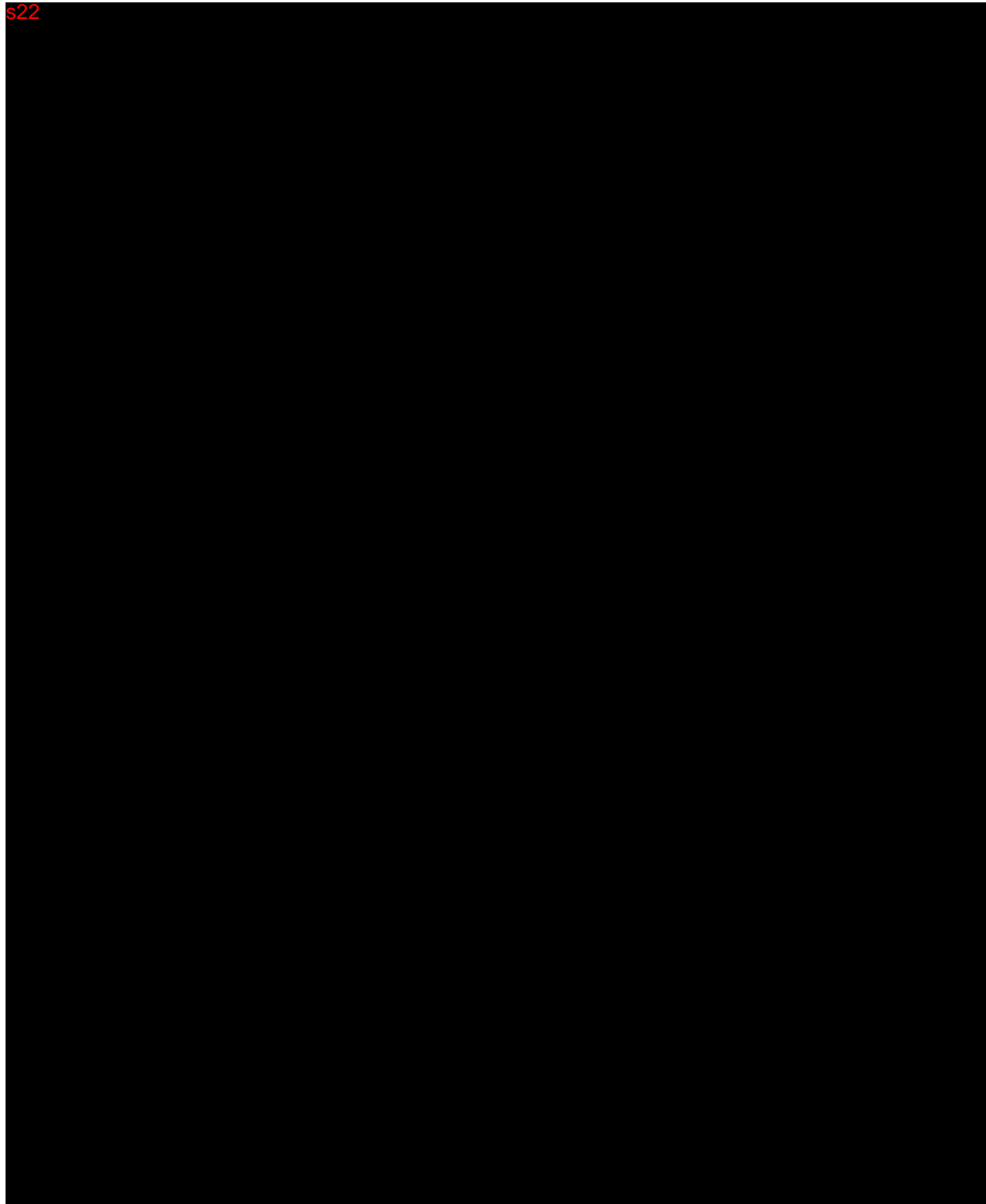
## HPLC General Worksheet

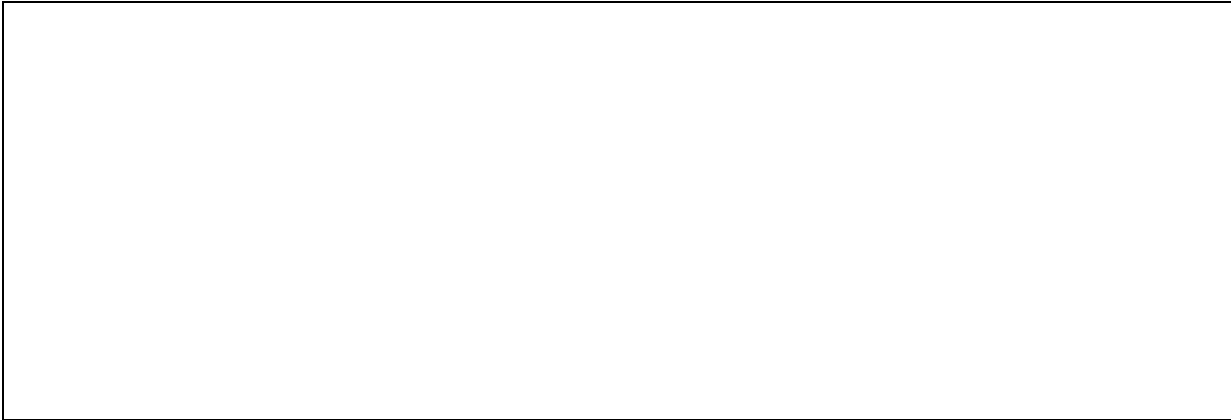
TEST DETAILS			
TEST NAME	Analysis of mRNA purity in Moderna SpikeVax bivalent vaccine by Size-based RPIP		
METHOD REFERENCE	Bio-BPC-Method-33 (Moderna SOP-1142)		
METHOD MODIFICATIONS (if any)	N/A		
MODIFICATIONS APPROVED BY:	N/A		
DATA LOCATION IN TRIM	E22-542283, D23-5194654		
NAME OF ANALYST	s22	TEST DATE	8/03/2023

BUFFERS AND SOLUTIONS	
SOLUTIONS	BATCH No:
Mobile phase A	CP08Mar23-1
Mobile phase B	CP08Mar23-2
SAMPLE DILUENT	N/A
Click or tap here to enter text.	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.

PIPETTES USED AND EXPIRY DATES	
LIMS# 33440	21/05/2023
LIMS# 33367	3/04/2023
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Click or tap here to enter text.	Click or tap to enter a date.

s22





<b>SYSTEM SUITABILITY CRITERIA AND RESULTS</b>			
<b>PARAMETERS</b>	<b>LIMITS</b>	<b>RESULTS</b>	<b>COMMENTS</b>
No peak in the water blank injection prior to the Sensitivity Solution	No peak	No peak	Pass
All other carryover water blank injections in the sequence interfere with the peaks of interest as compared to the total peak area of the mRNA working reference standard	Interference <=1%	Interfering peaks <1%	Pass
Signal to Noise ratio of Sensitivity Solution	>=10	14.74	Pass
% Recovery of the main peak area in each bracketing standard compared to the peak area of the mRNA working reference standard	90-110%	100.6	Pass
% Agreement of the main peak RT in each bracketing standard compared to the main peak RT of the mRNA working reference standard	90-110%	97.90	Pass
Absolute Difference of the Main Peak % Area for duplicate sample preparations – LIMS# 2303001127	<=5%	0.017	Pass
Absolute Difference of the Main Peak % Area for duplicate sample preparations – LIMS# 2303001129	<=5%	-1.851	Pass
Absolute Difference of the Main Peak % Area for duplicate sample preparations – LIMS# 2303001131	<=5%	0.563	Pass
Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.

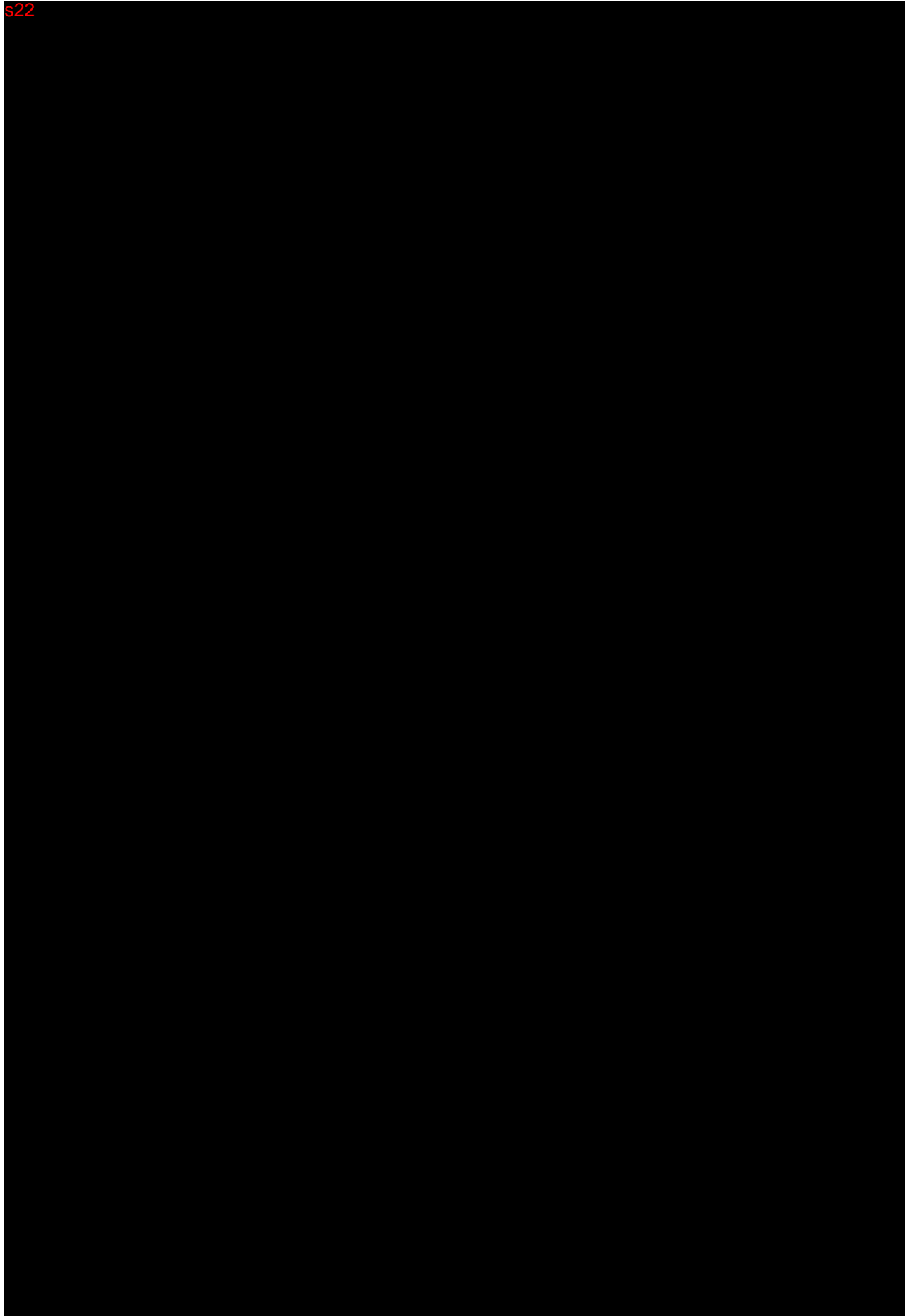


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<b>SAMPLE DETAILS</b>					
SAMPLE NAME	SPIKEVAX BIVALENT ORIGINAL/OMICRON BA.4-5 (elasomeran/davesomeran) COVID-19 VACCINE 0.1 mg/mL suspension for injection pre-filled syringe				
LIMS No:	2303001131	BATCH No:	0000027		
<b>SAMPLE DILUTIONS, CALCULATIONS</b>					
<b>Initial Conc.</b>	<b>Vol. sample</b>	<b>Vol. diluent</b>	<b>Final Conc.</b>	<b>DF</b>	<b>Inj. Vol.</b>
0.1 mg/mL	-	-	0.1 mg/mL	-	10 uL
100 uL of neat sample was transferred into two high recovery UPLC vials.					

<b>TEST RESULTS</b>			
PARAMETERS	LIMITS	RESULTS	COMMENTS
IG: RNA Fragments	s47	s47	
IG: Lipid adducts			
Main peak			
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**SAMPLE DETAILS**

SAMPLE NAME	Click or tap here to enter text.		
LIMS No:	Click or tap here to enter text.	BATCH No:	Click or tap here to enter text.

**SAMPLE DILUTIONS, CALCULATIONS**

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
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Click or tap here to enter text.

**TEST RESULTS**

PARAMETERS	LIMITS	RESULTS	COMMENTS
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**SAMPLE DETAILS**

SAMPLE NAME	Click or tap here to enter text.		
LIMS No:	Click or tap here to enter text.	BATCH No:	Click or tap here to enter text.

**SAMPLE DILUTIONS, CALCULATIONS**

Initial Conc.	Vol. sample	Vol. diluent	Final Conc.	DF	Inj. Vol.
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Click or tap here to enter text.

**TEST RESULTS**

PARAMETERS	LIMITS	RESULTS	COMMENTS
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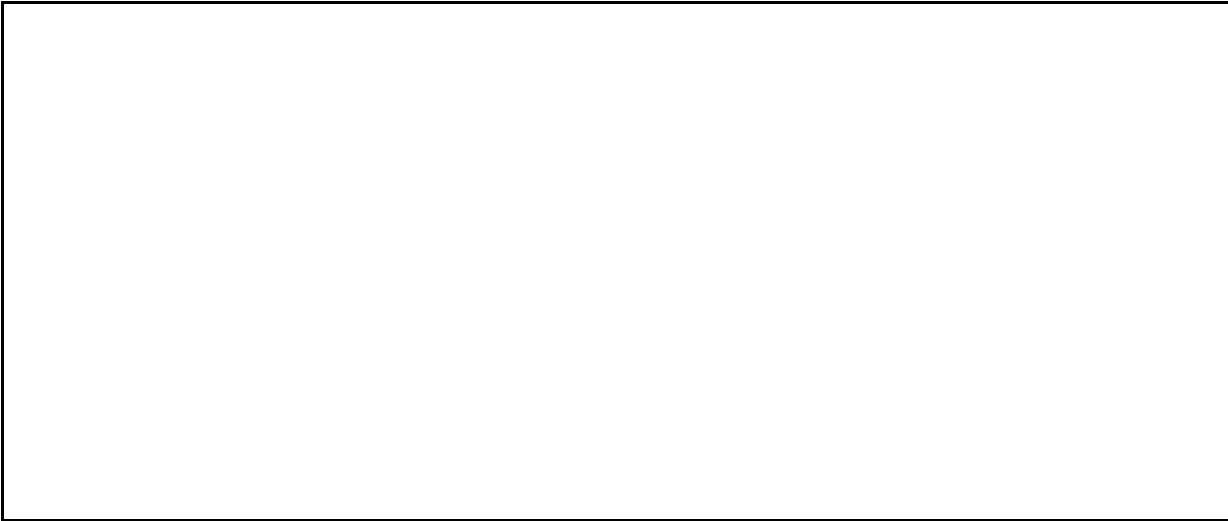
**NOTES**

Absolute % peak difference of main peak:

2303001127:  $83.511 - 83.494 = 0.017 \%$

2303001129:  $83.524 - 83.375 = -1.851 \%$

2303001131:  $82.631 - 82.068 = 0.563 \%$







Sample Set Summary Report

Sample Set: Spikevax 23030001127 08Mar23

Sample Set Information

Project Name: Biochemistry\2023\Spikevax - Purity  
Sample Set Name: Spikevax 23030001127 08Mar23  
Sample Set Acquired By: s22  
Start Date: 8/03/2023 3:13:26 PM AEDT  
Finish Date: 9/03/2023 3:33:51 AM AEDT  
Run Time: 31.00 Minutes  
Sample Set Altered: Yes  
SampleName: 2303001127, 2303001129, mRNA Std B, Conditioning, Conditioning H2O, Water blank SST, Water blank, Sensitivity, 2303001131, mRNA Std A, Water blank  
Acq Method Set: Spikevax RPIP\_2022\_MS  
Instrument Method Name: Spikevax RPIP\_2022\_IM  
Sample Set Method: !QuickSet

System Information

System Name: System 3  
Empower Node: Ucdp1bxnrk2  
Analytical\_Column\_1: ID#518 ProSwift RP-1S 4.6x50mm Sn002703  
Analytical\_Column\_2:

Processing Information

Processing Method: Spikevax RPIP 2022\_v3  
Processed By: s22/Biochem  
Processing Method Id: 1897  
Date Processed: 9/03/2023 9:34:39 AM AEDT, 9/03/2023 9:34:41 AM AEDT, 9/03/2023 9:34:43 AM  
Processing Node: Uclp4v71dk3  
Result Set Name: Spikevax 23030001127 08Mar23  
Result Set Date: 9/03/2023 9:34:36 AM AEDT  
Result Set Id: 2010  
Channel Description: PDA Ch1 260nm@4.8nm

Reporting Information

Report Method Name: Sample Set Summary Report  
Reported by: s22  
Print Date: 9/03/2023  
Time: 10:23:34 AM Australia/Canberra

## Injection Sequence Summary

	SampleName	Sample Type	Vial	Inj #	Run Time (Minutes)	Injection Volume (ul)	Sample Weight	Dilution	Level	Label
1	Conditioning	Control	1:E,1	1	31.00	10.00	1.00000	1.00000		
2	Conditioning	Control	1:E,1	2	31.00	10.00	1.00000	1.00000		
3	Conditioning	Control	1:E,1	3	31.00	10.00	1.00000	1.00000		
4	Conditioning	Control	1:E,1	4	31.00	10.00	1.00000	1.00000		
5	Conditioning	Control	1:E,1	5	31.00	10.00	1.00000	1.00000		
6	Conditioning H2O	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
7	Conditioning H2O	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
8	Conditioning H2O	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
9	Conditioning H2O	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
10	Water blank SST	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B0
11	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B1
12	Sensitivity	Control	1:E,3	1	31.00	10.00	1.00000	1.00000		A
13	mRNA Std A	Standard	1:A,1	1	31.00	10.00	1.00000	1.00000		S0101
14	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B2
15	2303001127	Unknown	1:A,2	1	31.00	10.00	1.00000	1.00000		U0101
16	2303001127	Unknown	1:A,3	1	31.00	10.00	1.00000	1.00000		U0101
17	2303001129	Unknown	1:A,4	1	31.00	10.00	1.00000	1.00000		U0102
18	2303001129	Unknown	1:A,5	1	31.00	10.00	1.00000	1.00000		U0102
19	2303001131	Unknown	1:A,6	1	31.00	10.00	1.00000	1.00000		U0103
20	2303001131	Unknown	1:A,7	1	31.00	10.00	1.00000	1.00000		U0103
21	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		
22	mRNA Std B	Standard	1:A,8	1	31.00	10.00	1.00000	1.00000		S0201
23	Water blank	Control	1:E,2	1	31.00	10.00	1.00000	1.00000		B2



<b>Type:</b> Biotherapeutics\BEE\Forms	<b>Number:</b> Bio-BEE-Form-39 / <b>Version:</b> 3
<b>Owner:</b> s22	<b>Approver:</b> s22
<b>Active:</b> 10/05/2022	<b>Review:</b> 10/11/2023
<b>Title:</b> Endotoxin Routine Assay Worksheet	

## Endotoxin Routine Assay Worksheet

Assay ID: 06Mar2023 Operator: s22

### Limulus Amoebocyte Lysate (LAL)

Lysate batch and expiry recorded on software for each assay

Ensure sensitivity of LAL batch has been confirmed. 'Lysate preparation details' shown below

### Recombinant Factor C (rFC)

rFC Enzyme, Fluorogenic Substrate & rFC Assay Buffer batches and expiry dates recorded on software for each assay

Ensure sensitivity of the rFC batch has been confirmed. 'rFC Reagent preparation details' shown below

### Control Standard Endotoxin (CSE) – refer to Bio-BEE-Method 5 and Bio-BEE-Form 37

CSE batch and expiry recorded on software for each assay

Reconstitution details for either KLAL or rFC – see Trim File D23-5103657

CSE Lot Number: 0001028884 Conc 50 EU/mL

### LAL Reagent Water (LRW) Lot Number: 0000981166 Expiry: 16Sep2023

How many samples were linked to this assay? 6

**This form is used for recording the assay details and results and only gives the method in point form. See Bio-BEE-SOP 28 and appropriate method for the detailed procedure.**

To avoid endotoxin contamination, use careful technique and **pyrogen free** equipment.

### Preparation of Assay

- Fill out the appropriate forms from the Quality Management System (QMS)
- Retrieve the required kit reagents from cold storage to equilibrate to room temperature
- Turn on plate reader and computer and follow the steps as detailed in Bio-BEE-Method 4

### Preparation of CSE (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare CSE as detailed in Bio-BEE-Method 5. CSE dilutions can be dispensed to the plate as they are prepared to save mixing time

- CSE is set up as in the table below - record %CV results from the final report

#### For a KLAL (KQCL) assay

Concentration	Plate wells ID	% CV
50 EU/ml	F1 – F2	0.93
5 EU/ml	E1 – E2	0.43
0.5 EU/ml	D1 – D2	0.10
0.05 EU/ml	C1 – C2	0.06
0.005 EU/ml	B1 – B2	0.00
Blank	A1 – A2	

#### For an rFC assay

Concentration	Plate wells ID	% CV
5 EU/ml	E1 – E2	N/A
0.5 EU/ml	D1 – D2	N/A
0.05 EU/ml	C1 – C2	N/A
0.005 EU/ml	B1 – B2	N/A
Blank	A1 – A2	

- Dispense 100 µl of the appropriate dilution of CSE into the appropriate wells of the plate.
- Continue with procedure as per the appropriate method

#### Preparation of Samples (refer to Bio-BEE-SOP 28 and appropriate Method)

- Prepare sample dilutions as in Bio-BEE-Form 42
- Pipette 10 µl of the 5 EU/ml standard to the appropriate PPC wells as per the plate layout
- Dispense 100 µl of the final sample dilutions into the 4 appropriate wells as per the plate layout

#### Starting the Assay

- The plate is then ready for the reaction. Prepare the software as set out in the Bio-BEE-Method 4
- “Run” the Template prepared earlier. Follow the prompts to the Pre-warming step

#### If performing a KLAL assay

- Prepare the required lysate vial/s (Bop-BEE-Method 6) and pour into the reagent reservoir

Lysate Preparations Details -

Lysate Lot Number	YL002L2U24	Expiry 11Jul2024
Date sensitivity confirmed	09Feb2023	TRIM#: E23-509118
Reconstitute lysate with	2.6mL (3x867µL)	mL of LAL Reagent Water (LRW)
Date reconstituted	06Mar2023	Combined with vial that reconstituted on 27/02/2023, use by 13/03/2023 (1.2mL)
Operator(s)	s22	1 vial
Use by date	N/A	(Lonza KQCL- 14 days from reconstitution, at below -10°C)

**OR**

*If performing an rFC assay*

- Make up the required volume of rFC reagent directly into the reservoir (Bio-BEE-Method 6)

rFC Reagent Preparations Details – (Note: once prepared, working reagent cannot be stored)

	Lot	Expiry	Volume
Date sensitivity confirmed	N/A	N/A	
Fluorogenic Substrate	N/A	N/A	N/A µL
rFC Assay Buffer	N/A	N/A	N/A µL
rFC Enzyme Solution	N/A	N/A	N/A µL

- Open cover – if using the Spectramax use the software to open and close the drawer
- Add 100 µl of either lysate (KLAL) **OR** working reagent (rFC) to each of the assay wells, carefully, and as quickly as possible
- Close the drawer on the plate reader and click OK to start the run. **Do not open drawer**

**Acceptance Criteria – for KLAL**

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>-0.999</u>
Slope between -0.400 and -0.100	<u>-0.228</u>
Y intercept between 2.500 and 3.500	<u>3.092</u>
Mean reaction times of blank $\geq$ mean reaction times of lowest standard	<u>Yes</u>
Coefficient of variation (CV) values for all standards are $< 10\%$	<u>Yes</u>
Were all acceptance criteria for the standard curve met?	<u>Yes</u>

## Acceptance Criteria – for rFC

The CSE standard curve must meet the following parameters for assay to be valid	
Correlation coefficient (r) absolute value $\geq 0.980$	<u>N/A</u>
Slope between 0.760 and 1.110	<u>N/A</u>
Y intercept between 2.500 and 5.000	<u>N/A</u>
Mean RFU of blank $\leq$ mean RFU of lowest standard	<u>N/A</u>
Coefficient of variation (CV) values for all standards are $< 25\%$	<u>N/A</u>
Were all acceptance criteria for the standard curve met?	<u>N/A</u>

## Conclusions

Follow procedures for 'Recording Results' detailed in Bio-BEE-Method 7

## Notes

Checked **s22** 7Mar2023