



## Compositional guideline for *Streptococcus salivarius* K12

### Name of the ingredient

*Streptococcus salivarius*

### Definition of the ingredient

*Streptococcus salivarius* is a gram positive bacterium found as part of the normal human oral microflora, predominantly colonising the tongue. *Streptococcus salivarius* K12 (isolated from the saliva of a healthy child) is a probiotic intended for use in oral cavity. The ingredient is prepared by fermentation of culture of *Streptococcus salivarius* K12. The cell concentrate is mixed with lyoprotectant, freeze-dried and milled into powder. The quality and safety of the probiotic *Streptococcus salivarius* was assessed for the strain K12 and the specific requirements listed below are strain specific.

**Table 1: Ingredient specific requirements**

Test	Method reference	Acceptance criteria
<b>Description</b>		
Appearance	Visual evaluation	Free flowing off-white powder
Odour/taste	Organoleptic evaluation	Proteinaceous taste
<b>Characteristics</b>		
Particle size	Sieve analysis	90% <500µm
Water activity (aw)	USP <1112> <sup>1</sup>	< 0.25

<sup>1</sup> United States Pharmacopoeia – National Formulary (USP-NF) General Chapter <1112>: APPLICATION OF WATER ACTIVITY DETERMINATION TO NONSTERILE PHARMACEUTICAL PRODUCTS.

Test	Method reference	Acceptance criteria
<b>Identification</b>		
Microscopic morphology	USP <1113> Gram staining <sup>2</sup>	Gram reaction: positive Cellular shape: cocci in chains or pairs
Macroscopic morphology	Visual examination of growth on Mitis salivarius agar at 37°C in 5% CO <sub>2</sub> in air after 24-48 hr	Colony size: 1-2 mm in diameter Colony shape: <ul style="list-style-type: none"> <li>Form: round</li> <li>Margin: entire</li> <li>Elevation: convex</li> </ul> Colony colour: blue Colony texture: mucoid
	Visual examination of growth on Blood agar [Columbia Agar Base with 5% human blood] at 37°C in 5% CO <sub>2</sub> in air after 24-48 hr	Colony size: <1 mm in diameter Colony shape: <ul style="list-style-type: none"> <li>Form: round</li> <li>Margin: entire</li> <li>Elevation: convex</li> </ul> Colony Colour: white Colony Texture: butyrous Haemolysis: None
Biochemical profile	USP <1113> biochemical tests <sup>2</sup> And/Or Automated microbial identification test (e.g. API 20 Strep test system)	Negative for: catalase Positive for: acetoin production, β-glucosidase, alkaline phosphatase, leucine aminopeptidase, D-lactose, D-trehalose, inulin, and D-raffinose.

<sup>2</sup> United States Pharmacopoeia – National Formulary (USP-NF) General Chapter <1113>: MICROBIAL CHARACTERIZATION, IDENTIFICATION, AND STRAIN TYPING.

Test	Method reference	Acceptance criteria
Molecular identification of strain	Barretto, Alvarez-Martin <i>et al.</i> 2012 <sup>3</sup>	Matches the sequence for <i>S. salivarius</i> K12
Deferred antagonism, P-typing producer	Wescombe <i>et al.</i> 2006 <sup>4</sup>	Matches the profile for <i>S. salivarius</i> K12
Antibiotic susceptibility profile	Clinical and Laboratory Standards Institute (CLSI) methods <sup>5</sup>  Or  Automated antimicrobial susceptibility testing system (e.g. Vitek 2 AST)	Susceptible to: penicillin, amoxicillin, tetracycline and erythromycin
Streptococcal virulence genes	Burton <i>et al.</i> 2006 <sup>6</sup>	Absence of representative streptococcal virulence genes: <i>emm</i> , <i>scpA</i> , <i>speB</i> , <i>smez-2</i> , and <i>sagA</i>
<b>Assay</b>		
<i>Streptococcus salivarius</i> K12 enumeration	Viable plate count on CAB K12 agar cultured at 37°C in 5% CO <sub>2</sub> in air after 24-48 hr per Ishijama 2012 <sup>7</sup> , Burton <i>et al.</i> 2011 <sup>8</sup>	> 1 x 10 <sup>11</sup> CFU/g

<sup>3</sup> Barretto, C. & Alvarez-Martin, P. (2012). Genome sequence of the lantibiotic bacteriocin producer *Streptococcus salivarius* strain K12. *J Bacteriol*, 194(21), pp. 5959-5960.

<sup>4</sup> Wescombe *et al.*, *AEM*, (2006) p. 1459–1466. Production of the Lantibiotic Salivaricin A and Its Variants by Oral Streptococci and Use of a Specific Induction Assay To Detect Their Presence in Human Saliva

<sup>5</sup> CLSI. *Performance Standards for Antimicrobial disk Susceptibility Tests*. 13<sup>th</sup> ed. CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

<sup>6</sup> Burton, J. P., Wescombe, P. A., Moore, C. J., & Chilcott, C. N. (2006). Safety assessment of the oral cavity probiotic *Streptococcus salivarius* K12. *Appl Environ Microbiol*, 72(4), 3050–3053.

<sup>7</sup> Ishijima, S.A., Hayama, K., Burton, J.P., Reid, G., Okada, M., Matsushita, Y., Abea, S. (2012) Effect of *Streptococcus salivarius* K12 on the *In Vitro* Growth of *Candida albicans* and Its Protective Effect in an Oral Candidiasis Model. *Appl Environ Microbiol*. 78(7):2190 - 9

<sup>8</sup> Burton, J.P., Cowley, S., Simon, R. R., McKinney, J., Wescombe, P.A., Tagg J.R (2011) Evaluation of safety and human tolerance of the oral probiotic *Streptococcus salivarius* K12: A randomized, placebo-controlled, double-blind study. *Food and Chemical Toxicology*.

## **Incidental metals and non-metals**

While ingredient manufacturers are encouraged to include limits for incidental metals and non-metals, it is the product into which those substances are formulated that contains the ingredient, alone or in combination with other ingredients, which must comply with the acceptance criteria set in the United States Pharmacopoeia – National Formulary (USP-NF) general chapter '<2232> Elemental Contaminants in Dietary Supplements'.

## **Microbiology**

While substance manufacturers are encouraged to include limits for objectionable microorganisms, it is the product into which those substances are formulated that is subject to a legally binding set of criteria. The [Therapeutic Goods Order No. 77](#) '*Microbiological Standards for Medicines*' mandates that any finished product that contains the ingredient, alone or in combination with other ingredients, must comply with the microbial acceptance criteria set by Clause 9 of the Order.

### **Key to abbreviations:**

BP = British Pharmacopoeia

Ph. Eur. = European Pharmacopoeia

USP = United States Pharmacopoeia

CFU = Colony forming units