Australian Government
Department of Health
Therapeutic Goods Administration

Compositional Guideline for *Euglena gracilis* whole cell dry

Name of the ingredient

Euglena gracilis whole cell dry

Definition of the ingredient

Euglena gracilis is a single-celled, spindle-shaped/elongated, filamentous microalgae belonging to the *Euglenaceae* family. It consists of a single nucleus, chloroplasts with pigments (when grown in light) or proplastids (when grown in the dark), a contractile vacuole, an eyespot, and a single flagellum. Under microscope it appears as a thick-walled oblong-shaped organism. The ingredient is a dried powder of the whole-cell algae fermentate produced through a patented fermentation process (Patent No. US 9,574,217 B2) containing a minimum of 50% algae *beta*-1,3-glucans.

Table 1. Ingredient specific requirements

Test	Method reference	Acceptance criteria
Description		
Appearance	Visual	Free flowing powder, free of foreign material
Colour	Visual	Yellow-Tan
Odour	Organoleptic	Characteristic of algae
Characteristics		
Moisture	Ohaus MB45 Moisture Analyser	≤6%
Protein	Dumas	≥15%

Total Fatty acids	AOAC 922.06, 954.02, 933.05, 925.32	≤15%
Ash	AOAC 923.03	≤10%
Identification		
Cellular morphology	Microscopic examination	Identification of paramylon (*beta*-(1,3)-glucan) granules within characterisitic *Euglena gracilis* cells
Genotypic identity	rDNA sequencing	Conforms to reference DNA-sequence for *Euglena gracilis* on HERB™ database.
Assay		
beta-1,3-glucan	AOAC 991.43	≥50%

Table 2. Incidental constituents

Test	Method reference	Acceptance criteria
Residual Solvents		
The ingredient is produced without the use of extraction solvents.		
Incidental metals and non-metals		
Lead	AOAC 2011.19, AOAC 993.14	≤0.5 mg/kg (ppm)
Cadmium	AOAC 2011.19, AOAC 993.14	≤0.5 mg/kg (ppm)
Mercury	AOAC 2011.19, AOAC 993.14	≤0.05 mg/kg (ppm)
Arsenic	AOAC 2011.19, AOAC 993.14	≤0.02 mg/kg (ppm)

Pesticide residues and environmental contaminants: (including agricultural and veterinary substances)		
Mycotoxins (Total Aflatoxins: sum of B1, B2, G1, G2)	UHPLC-MS/MS[1]	≤1 mg/kg
PAHs: Benzo(a)pyrene	GC-MS[2]	≤10 µg/Kg (ppb)[3]
PAHs: Sum of benzo(a)-pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene.	GC-MS[2]	≤50 µg/Kg (ppb)[3]
Microbiology		
Aerobic plate count	AOAC 966.23	≤10,000 CFU/g
Coliforms	FDA-BAM	≤100 MPN/g
Yeast and Mould	FDA-BAM	≤500 CFU/g
Escherichia coli	USP <62>	Negative/10 g
Staphylococcus aureus	USP <62>	Negative/10 g
Salmonella	USP <62>	Negative/25 g
Listeria monocytogenes	AOAC 2004.06	Negative/25 g

Footnotes:

1. Varga, E., Glauner, T., Koppen, R., Mayer, K., Sulyok, M., Schumacher, R., Krska, R. and Berthiller, F., Stable isotope dilution assay for the accurate determination of mycotoxins in maize by UHPLC-MS/MS, *Analytical and BioAnalytical Chemistry*, 402:2675-2686 (2012).
2. Residues quantified using Triple Quad Mass-Spectrometry. Compounds analysed include the following: Benz(a)anthracene, Benzo(a)pyrene, Benzo(b)fluoranthene, Benzo(g,h,i)perylene, Benzo(k)fluoranthene, Chrysene, Dibenz(a,h)anthracene, Indeno(1,2,3-c,d)pyrene, Pyrene.
3. Limits are based on EU directive Commission Regulation (EC) No 1881/2006 and amendments for food supplements.

Key to abbreviations:

AOAC = Association of official analytical collaboration
BAM = Bacteriological Analytical Manual
FDA = Food and Drug Administration
ICP = Inductively Coupled Plasma
ISO = International organisation for standardisation
MS = Mass spectrometry
PAHs = Polycyclic Aromatic Hydrocarbons
UHPLC = Ultra High-pressure liquid chromatography

Version	Description of change	Effective date
1	Original	1/3/2022