



Certificate of Analysis

This certificate is designed in accordance with ISO Guide 31 [1].

Product Name: **Clostridium sporogenes WDCM 00008 Vitroid™**
Product Number: VT000082-10EA
Lot: **LRAB4145**
Expiration Date: December 31, 2019
Storage Conditions: Store at -20 °C
Biosafety Level: 1
Color of discs: Light Yellow
Passages: 2
Source: CECT 485

Certified values and uncertainties:

Enumeration medium / method	Medium / kit supplier	Procedure	Number of colony forming units (cfu)	
			Certified value ¹⁾	Expanded Uncertainty ¹⁾
m-CP Agar	SIGMA-ALDRICH 75605	A)	69.0	6.23
Tryptic Soy Blood Agar (TSA+B)	Sigma-Aldrich 22091	B)	83.0	6.92

Procedures:

A) The test values were obtained by diluting a disc in 100mL of phosphate buffer, filtering a fraction of the volume, and placing filtrate on the agar plate (see below), and incubating under aerobic conditions at 44.0±0.5 °C for 24±2 hrs on HiCrome mCP Agar (Sigma-Aldrich 75605).

B) The test values were obtained by diluting a disc and spreading 40µL on the agar plate with a loop (see below) after leaving to rehydrate (10 to 15 minutes), and incubating under aerobic conditions at 37.0±0.5 °C for 24±2 hrs on Tryptic Soy Blood Agar (Sigma-Aldrich 22091).

This certified reference material was designed, produced and verified for accuracy and stability in accordance with ISO/IEC 17025 [2] and ISO Guide 34 [3].

I) The certified value represents the unweighted mean cfu per disc value from a statistically relevant number of samples covering the entire production lot. The characterization uncertainty μ (characterisation) represents the dispersion of measurement values, calculated as standard deviation.

II) The combined standard uncertainty, μ (CRM), is calculated as the square root of the sum of squares of the individual contributions, according

$$\text{to: } \mu_{CRM} = \sqrt{\mu_{char}^2 + \mu_{homogeneity}^2 + \mu_{stability}^2}$$

The expanded uncertainty, U(CRM) is reported at the 95% confidence level with a coverage factor k=2: U(CRM) = μ (CRM) * k.

Description:

Vitroids™ are disc-shaped, microbiological reference materials. Each disc contains a quantified number of microorganisms (colony forming units; cfu), immobilized in a solid water soluble matrix. The discs are sensitive to moisture and supplied in airtight polypropylene tubes



Source: CECT 485
containing a dessicant tablet in the cap.

Intended use:

Vitroids™ are suitable for quality controls of culture media, antibiotics, disinfectants, for process and internal quality controls, for food, beverage and environmental testing, as well as for method development and validation in general.

The discs can be used in combination with any medium suitable for the microorganism provided in Vitroids™ disc format and with any volume; however, please note, the certified colony forming units (cfu) value is method and media specific. If Vitroids™ are to be used for media and methods other than those stipulated above in the certified values section, you are advised to calibrate the discs for the conditions in your own laboratory.

Storage and handling:

Store the Vitroids™ tubes unopened at -20°C until final use. (Once a tube has been opened, the enclosed disc should be used within 30 days). Vitroids™ are water soluble and therefore easily reconstituted:

- 1) Remove the tube(s) to be used from the freezer and allow the disc(s) to reach ambient temperature (5 to 10 minutes) before use. Do not refreeze, and use the disc(s) within one hour of transfer to ambient temperature.
- 2) Open the tube and remove the Vitroid™ by inverting the tube over the medium to be used. Vitroids™ can be re-hydrated on solid medium or in liquid medium or buffer, respectively. Please do not re-hydrate the discs directly in distilled water. Instead, re-hydrate in a small volume of buffer (e.g. maximum recovery diluent), and transfer the entire volume into distilled water.
- 3) Leave at room temperature to re-hydrate (for 10 to 15 minutes) and ensure that the disc is completely dissolved.
- 4) Solid media: spread the resulting drop with a loop over the entire plate. Liquid media: mix thoroughly.
- 5) Test and incubate the sample in accordance with routine enumeration procedures.

Stability:

Long term stability is analyzed by re-testing samples stored at the recommended storage temperature every 4 months over the entire shelf life using the same methods as mentioned in the certified values section. Short term stability is analyzed by the simulation of product shipment at elevated temperatures. The uncertainty related to the stability of the product, μ (stability) is comprised of the uncertainty of short term stability and the uncertainty of long term stability and an integral part of the expanded uncertainty stipulated in the certified values section.

Homogeneity:

Homogeneity is tested and confirmed by analysis of a statistically relevant number of samples covering the entire production lot by means of statistical models (e.g. ANOVA). The uncertainty related to the homogeneity of the product, μ (homogeneity) is an integral part of the expanded uncertainty value stipulated in the certified values section.

Hazardous information:

Please refer to the Safety Data Sheet (available online: <http://go.sigmaaldrich.com/vitroids>).

References:

- [1] ISO Guide 31, Reference materials - Contents of certificates and labels
- [2] ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories
- [3] ISO Guide 34, General requirements for the competence of reference material producers
- [4] ISO 7218, Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations



Robert O'Brien - QC Supervisor

Certification Date February 02, 2017

Version 1754-222017



Mark Pooler - QA Supervisor