

Specification

Staining solution in Gram Staining

Presentation

1 Prepared bottle
 Bottle 1000 ml
 with: 1000 ± 10 ml

Packaging Details

1 plastic bottle 1L capacity

Shelf Life

60 months

Storage

8-25 °C

Composition

Composition (g/l):

Crystal Violet..... 5.0 g

Distilled water..... 1000.0 ml

Description /Technique

This solution has been prepared according to the Gram staining, and it is very stable.

1. Fix the smear following the habitual method and let it cool.
2. Cover the extension with Crystal Violet Dye Solution and let it act for 1 minute.
3. Wash the dye excess. The best way is to put the preparation in a precipitate glass with fluent water. Do not wash excessively. This step may be critical for the rest of the test.
4. Cover the preparation with Lugol Solution for 1 minute.
5. Wash softly again with tap water for approx. 5 minutes.
6. Decolourise, pouring the Gram Decolourizer, drop to drop, over the slanted slide until total decolourising. Any case, this step may not be longer than 60 seconds.
7. Wash with water to stop the decolouring action.
8. Cover the preparation with Safranin Dye Solution and let it act for 1 minute.
9. Wash gently to remove the excess of dye, with tap water.
10. Dry and observe under microscope in homogeneous immersion.

Micro organisms that get coloured by the first dye, crystal violet, become dark blue (or violet) coloured and it is said that they take the Gram, and they are called "Gram positive" (G+). Those micro organisms that just get coloured by the contrast dye become red and they are called "Gram negative" (G-).

Most of eukaryote cells, except yeast, are coloured as Gram negative and thus the staining is not very significative. In spite of, it is one of the first levels in the systematic identification of prokaryote: between the bacteria, all the cocci, except *Neisseria* and *Veillonella*, are Gram positive, and all the sporogenic bacilli and some part of the other bacilli (acid lactic bacteria and propionibacteria) are Gram positive too. Spiriles, vibria, rikettsia, clamidia and most bacilli are Gram negative.

Quality control

Physical/Chemical control

Color : Violet

pH: at 25°C

Microbiological control

Not applicable

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Gram stain to the following microorganisms:

Microorganism

Escherichia coli ATCC® 25922, WDCM 00013

Staphylococcus aureus ATCC® 6538, WDCM 00032

Growth

Gram negative (Reddish)

Gram positive (Purple-violet)

Sterility Control

Not Performed - Staining solution without nutrients.

Reference: 911517ZA **Technical Data Sheet**

Product: **CRYSTAL VIOLET GRAM**



Bibliography

- BARTHOLOMEW, J. W. (1962) Variables Influencing Results, and the Precise Definition of Steps in Gram Staining as a Means of Standardizing the Results Obtained. Stain Technol. 37:139-155.
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- PAIK, G. (1980) Reagents, Stain and Miscellaneous Procedures. In "Manual of Clinical Microbiology". Lennette, Balows, Hausler y Truant. Eds. ASM. Washington.