**Reference:** 911540ZA **Technical Data Sheet**

**Product:** **SAFRANIN GRAM**

## Specification

Staining solution in Gram Staining

## Presentation

| | Packaging Details | Shelf Life | Storage |
|---|---|---|---|
| 1 Prepared bottle<br>Bottle 1000 ml<br>with: 1000 ± 10 ml | 1 plastic bottle 1L capacity. | 60 months | 8-25 ºC |

## Composition

Composition (g/l):

| | |
|---|---|
| Safranin | 10.0 g |
| Ethanol denatured | 100.0 mL |
| Distilled water | 900.0 mL |

## Description /Technique

Description:

The contrast dye is composed by the classic Safranin Solution. It is demonstrated that this solution is more effective than the fuchsine. This contrast dye is employed also in many other staining methods.

Technique:

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/Fucsin 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 coloured and it is said that they take the Gram, and they are call “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 : Reddish pH: at 25ºC

### Microbiological control

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

Gram stain to the following microorganisms:

Microbiological control according to ISO 11133:2014/A1:2018; A2:2020.

| Microorganism | Growth |
|---|---|
| *Escherichia coli ATCC® 25922, WDCM 00013* | Gram negative (Reddish) |
| *Staphylococcus aureus ATCC® 6538, WDCM 00032* | Gram positive (Purple-violet) |
| *Bacillus cereus ATCC® 11778, WDCM 00001* | Gram positive (Red stained sporangia) |

### Sterility Control

Not Performed - Staining solution without nutrients.

## 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.
·CLARK, G. (Ed.) (1981) Staining Procedures. 4th ed. William & Wilkins. Baltimore. MD.
·PAIK, G. (1980) Reagents, Stain and Miscellaneous Procedures. In "Manual of Clininal Microbiology". Lennette, Balows, Hausler y Truant. Eds. ASM. Washington.
. ISO 11133:2014/ Adm 1:2018/ Adm 2:2020/ Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.