

GRAM SAFRANINE 5X CONCENTRATED

IVD In vitro diagnostic medical device

Safranine O stock solution for preparing contrast staining solution acc. to Gram INSTRUCTIONS FOR USE

REF Catalog number: S005X-0T-100 (100 mL)

S005X-0T-500 (500 mL)

S005X-0T-1L (1000 mL)

Introduction

Gram Safranine 5X concentrated stock solution is used for bacterial Gram staining. The solution must be diluted before use and used for counterstaining Gram-negative bacteria. That enables differentiating the blue and purple-colored Gram-positive bacteria from the red-colored Gram-negative bacteria. Apart from use in bacteriology, Safranine 0 solution can also be used in histology and cytology because it stains cellular nuclei red. It is used for identification of cartilage, mucins, and mastocyte granules. It also enables identification of chondrocytes made from human and rodent mesenchymal stem cells.

Product description

• GRAM SAFRANINE 5X CONCENTRATED - Solution for counterstaining acc. to Gram.

Other preparations and reagents that may be used:

- · Primary differential Gram staining dye solution, such as BioGnost's Gram Crystal Violet 1% or Gram Crystal Violet 0.4% solution
- Iodine solution used in differentiating Gram staining, such as BioGnost's Gram Lugol solution, stabilized or Gram Lugol solution.
- Destaining solution used in differentiating Gram staining, such as BioGnost's Decolorization solutions 1, 2 or 3.
- Glass slides used in microbiology, such as VitroGnost ECONOMY GRADE or glass slides used in cytology, such as VitroGnost STANDARD GRADE or high quality
 glass slides used in histopathology, such as VitroGnost SUPER GRADE or one of more than 30 models of VitroGnost glass slides.
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparation of solutions

Working Gram Safranine solution

• Mix 20 ml of Gram Safranine 5x concentrated solution with 80 ml of distilled (demineralized) water.

Preparing the sample for staining

- Transfer the sample on a clean glass slide using a sterilized smear loop.
 Note: Bodily fluids, discharge, pus, and liquid or solid bacterial culture can be used as samples.
- Spread the sample evenly across the glass slide using 1-2 drops of saline solution.
- Fix the sample using the Bunsen burner after drying by wriggling the glass slide through the cone of flame for 2-3 times.
- · Cool the glass slide and begin the process of staining.

Sample staining procedure

1.	Staining with Gram Crystal Violet 1% solution	1 min
	Note: BioGnost's Gram Crystal Violet 1% solution can be used instead of Gram Crystal Violet 0.4% solution.	
2.	Pour excessive dye off the section	
3.	Rinse the section quickly using stabilized Gram Lugol solution.	
	Note: It is also possible to use (non-stabilized) Gram Lugol solution.	
4.	Fix the dye by treating the section using the stabilized Gram Lugol solution for	1 min
5.	Rinse the section carefully using distilled/demi water until all the iodine is removed. Pour excessive water off the	
J.	section.	
6.	Treat the preparation using Gram Decolorizer solution. End the process when the section turns grey-blue.	10-15 seconds
	Note: It is possible to use Gram Decolorizer solutions 1 and 3. Caution! Gram Decolorizer 3 solution reacts extremely quickly. By	
	overly treating with Decolorizer solution, the dye will be washed away from Gram-positive bacteria as well.	
7.	Rinse in distilled (demi) water	5 seconds
8.	Treat the preparation using working Gram Safranine solution.	1 min
9.	Rinse the section carefully with distilled (demi) water.	
10.	Dry the section using filter paper or let it dry by air.	
11.	Add 1 drop of Immersion oil on the section.	
12.	Examine the section under immersion lens.	

Result

Gram-positive bacteria - blue-purple Gram-negative bacteria - red

Note

Microbiology staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for use. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory. In order to avoid an erroneous result, a positive and negative check is advised before application.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet which is available on demand.

Storing, stability and expiry date

Keep Gram Safranine 5x concentrated in a tightly sealed original packaging at temperature between $+15^{\circ}$ C to $+25^{\circ}$ C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

- 1. Carson, F. L., Hladik, C. (2009): Histotechnology: A Self-Instructional Text, 3rd ed., Chicago: ASCP Press
- 2. Kiernan, J. A. (2008): Histological and Histochemical Methods, 4th ed., Bloxham: Scion Publishing Ltd.

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