GRAM CRYSTAL VIOLET, PHENOL FREE REAGENT

IVD *In vitro* diagnostic medical device CE

Solution for use in BioGram Eco kit
INSTRUCTIONS FOR USE

REF Catalogue number: GCF-OT-50 (50 mL) GCF-OT-250 (250 mL) GCF-OT-500 (500 mL) GCF-OT-1L (1000mL) GCF-OT-2.5L (2500mL)

Introduction

Gram Crystal Violet, phenol free reagent is a component of BioGram Eco kit used for differential staining between Gram-positive and Gram-negative bacteria. Gram staining is a method of differentiating bacterial species and it is commonly known and used in microbiology. It is also one of the most frequently used diagnostic methods in hospital and clinical laboratories. Gram staining differentiates bacteria into two groups: Gram-positive and Gram-negative. Division is based on the two groups' bacterial membrane structural differences, i.e. their capability of retaining the dye. BioGnost's Gram Lugol solution, stabilized, which is an aqueous solution of iodine and potassium iodide in its composition, also contributes to this ability. During the Gram staining process, it allows the dye to enter the bacterial cell and create insoluble complexes of iodine and primary dye. With this action, it enables color retention and later identification of Gram-positive bacteria.

Product description

- **GRAM CRYSTAL VIOLET, PHENOL FREE REAGENT** – reagent for use in microbiology for staining according to Gram

Other preparations and reagents that may be used:

- Gram Sodium bicarbonate, solution for preparing working solution
- Iodine solution for use in Gram differential staining procedures, such as BioGnost's Gram Lugol solution, stabilized
- Destaining solution for use in Gram differential staining processes, such as BioGnost Gram Decolorizer Solution 2
- Counterstain dye solution for use in differential Gram staining, such as BioGnost's Gram Safranin solution
- 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

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.

Preparing the working solution

In a separate container, mix Gram Crystal Violet, phenol free reagent and Gram Sodium bicarbonate, solution, in 1:1 ratio. The solution can be kept at room temperature for 2 days, and at +4°C for up to 4 days in a closed container. Calculate the required volume of working solution according to the amount of glass slides, if staining a single slide requires 3 ml of working solution.

Sample staining procedure

1.	Stain the section using working solution (cover the sample with the reagent completely)	**1 min**
2.	Gently rinse the section with distilled/demi water	**5 seconds**
3.	Apply 1-2 mL of Gram Lugol solution, stabilized on the section	**1 min**
4.	Gently rinse the section with 2 mL of distilled/demi water	**5 seconds**
5.	Treat the section with 1-2 mL of Gram Decolorizer solution 2. Gently shake the slide, stop the incubation after the thickest part of the sample ceases to release bluish dye	**5-10 seconds**
6.	Quickly rinse the section using 3-5 mL of distilled/demi water	**5 seconds**
7.	Treat the preparation using Gram Safranin solution	**15-30 seconds**
8.	Rinse the section carefully with distilled/demi water	**5 seconds**
9.	Dry the section by air or using thermostat	
10.	Apply Immersion oil and view under microscope	

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 Lugol solution in a tightly sealed original packaging at temperature of 15°C to 25°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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	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	European Conformity		BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until	LOT	Lot number		Manufacturer		
IVD	For *in vitro* diagnostic use only		Keep in dry place		Caution - fragile					CE	