GRAM DECOLORIZER 3 SOLUTION

IVD *In vitro* diagnostic medical device CE

Solution for use in Gram bacterial staining

INSTRUCTIONS FOR USE

REF Catalogue number: GD3-OT-100 (100 mL) GD3-OT-250 (250 mL) GD3-OT-500 (500 mL) GD3-OT-1L (1000 mL)

Introduction

BioGnost's Gram Decolorizer 3 solution is a transparent ethanol and acetone solution. It is used in microscopic microbiological diagnostics in processes of Gram staining. Gram Decolorizer 3 solution acts quickly and is suitable for staining methods that require rapid destaining or for use with staining machine.

Product description

- **GRAM DECOLORIZER 3 SOLUTION** - Acetone and ethanol solution that acts quickly and is used for Gram staining.

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 2% solution
- Iodine solution used in differentiating Gram staining, such as BioGnost's Gram Lugol solution, stabilized or Gram Lugol solution, stabilized.
- Counterstain solution for differentiating 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.
- Fixate 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.	Stain with Gram Crystal Violet 1% solution	1 min
2.	Pour excessive dye off the section.	
3.	• Rinse the section carefully using stabilized Gram Lugol solution.	
4.	Fix the dye by treating the section using stabilized Gram Lugol solution	1 min
5.	• Rinse the section carefully with distilled/demineralized water.	5 seconds
6.	• Treat the preparation using Gram Decolorizer 3 solution. • End the process when the section turns grey-blue.	5 seconds
	Note: Caution! Gram Decolorizer 3 solution reacts extremely quickly. If excessive amount of the solution is used the dye from Gram-positive bacteria will also be washed away.	
7.	• Rinse the section carefully with distilled/demineralized water.	5 seconds
8.	• Treat the preparation using Gram Safranin solution.	1 min
9.	• Rinse the section carefully with distilled/demineralized water.	5 seconds
10.	• Dry the section using filter paper or let it dry by air.	
11.	• Add a drop of immersion oil on the section (Cedar or Immersion oil).	
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. Acetone and ethanol contained in Gram Decolorizer 3 solution are highly flammable. Extreme caution is advised. Avoid breathing in the fumes and keep the bottle firmly sealed and away from the source of ignition.

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.

Storing, stability and expiry date

Keep Gram Decolorizer 3 solution in a tightly sealed original packaging at temperature of 15 to 25 °C. Do not keep in cold 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. Myers, Richard L. (2007): *The 100 Most Important Chemical Compounds: A Reference Guide*, Westport, Conn.: Greenwood Press

GD3-OT-X, V6-EN4, 21 February 2017, AK/VR

	Refer to the supplied documentation	°C	Storage temperature range		Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
	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						