BIOGNOST®

GRAM SODIUM HYDROGEN CARBONATE, SOLUTION

IVD In vitro diagnostic medical device CE

Reagent for use in BioGram Eco kit

INSTRUCTIONS FOR USE

REF Product code: GNHK-OT-100 (100 mL) GNHK-OT-250 (250 mL) GNHK-OT-500 (500 mL) GNHK-OT-1L (1000 mL) GNHK-OT-2.5L (2500 mL)

Introduction:

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. That division is based on the two groups' bacterial membrane structural differences, i.e. their capability of retaining the dye. Gram-positive bacteria have a thicker cellular membrane which enables retaining the dye inside the cell by treating them with iodine solution that creates insoluble iodine and primary dye complex. Gram-negative bacteria have thinner cellular membrane structure which cannot retain the dye. It washes away through the membrane and using counterstaining forms the basis for differentiating between the two bacteria groups. Gram Sodium hydrogen carbonate solution is a part of BioGnost's BioGram Eco kit which does not contain phenol and it minimizes exposure to harmful chemicals while retaining optimal staining and diagnostic results.

Product description:

- **GRAM SODIUM HYDROGEN CARBONATE, SOLUTION** - For differentiating bacteria according to Gram.

Example of using Gram Sodium hydrogen-carbonate, solution with BioGram Eco kit

Other preparations and reagents that may be used:

- 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 oils, such as Immersion oil and Immersion oils types 37, A, or FF

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.
- After drying on air, fix the sample using one of the following methods:

1. Using manual staining procedure, add a few drops of fixative on the sample and let it act for 1-2 minutes. Continue the staining procedure manually or in automatic stainer.
2. Fix the sample using Bunsen burner by wriggling the glass slide through the cone of flame for 2-3 times. Cool the glass slide and begin the process of staining.
3. During manual staining, fix the sample by applying a few drops of methanol. Let it act for 1-2 minutes and continue with either manual or automatic staining procedure.

Preparation of working solution

In a separate jar mix Gram Crystal Violet, phenol-free reagent and Gram Sodium hydrogen carbonate, solution in 1:1 ratio. The solution can be kept at room temperature for 2 days, and at +4°C for 4 days in a closed jar. Calculate the necessary volume of working solution according to number of slides (if staining one slide requires 3 ml of working solution).

Manual staining procedure

1.	Stain the sample using working solution (completely cover the sample using the reagent)	**1 min**
2.	Gently rinse the section with distilled/demineralized water.	**5 seconds**
3.	Apply 1-2 ml of Gram Lugol solution, stabilized on the sample	**1 min**
4.	Gently rinse the sample 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 stops releasing bluish dye	**5-10 seconds**
6.	Quickly rinse the sample with 3-5 ml of distilled/demi water	**5 seconds**
7.	Treat the preparation using Gram Safranin solution	**15-30 seconds**
8.	Gently rinse the section with distilled or demineralized water.	**5 seconds**
9.	Dry the section on air or in thermostat.	
10.	Add immersion oil and microscope the section	

Result

Gram-positive bacteria - blue-purple
Gram-negative bacteria - pink

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.

Storing, stability and expiry date

Keep Gram Sodium hydrogen-carbonate, solution in a tightly sealed original packaging at temperature of +15°C 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. 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						