BIOGNOST®

BIOGRAM 4 KIT

IVD *In vitro* diagnostic medical device CE

Four-reagent kit for identification of bacteria according to Gram in four steps
For differentiation of Gram-positive from Gram-negative bacteria

INSTRUCTIONS FOR USE

REF Catalogue number: BGR4-100T (for 100 tests) BGR4-K-100 (5x100 ml) BGR4-K-250 (5x250 ml) BGR4-K-500 (5x500 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. This 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 through counterstaining forms the basis for differentiating between the two bacteria groups. BioGnost's BioGram 4 kit contains Gram Crystal Violet 1% solution, stabilized Gram Lugol solution, two packages of Gram Decolorizer solution 2 and Gram Safranin solution. Its characteristics make it an optimal bacteria-staining agent which provides consistent results.

Product description:

BIOGRAM 4 KIT - Four-reagent kit in 5 packages for differentiating bacteria according to Gram in four steps

The kit contains:	5x30 mL (BGR4-100T)	5x100 mL (BGR4-K-100)	5x250 mL (BGR4-K-250)	5x500 mL (BGR4-K-500)
Gram Crystal violet 1% solution	30 ml (GC1-OT-30)	100 ml (GC1-OT-100)	250 ml (GC1-OT-250)	500 ml (GC1-OT-500)
Gram Lugol solution, stabilized	30 ml (GLS-OT-30)	100 ml (GLS-OT-100)	250 ml (GLS-OT-250)	500 ml (GLS-OT-500)
Gram Decolorizer solution 2	2x30 mL (GD2-OT-30)	2x100 mL (GD2-OT-100)	2x250 mL (GD2-OT-250)	2x500 mL (GD2-OT-500)
Gram Safranin solution	30 ml (GSF-OT-30)	100 ml (GSF-OT-100)	250 ml (GSF-OT-250)	500 ml (GSF-OT-500)

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 BioGnost's Immersion oil, Cedarwood oil, Immersion oils types 37, A, B, 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.
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 Gram Lugol solution, stabilized.	
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 solution 2. End the process when the section turns grey-blue.	10-15 seconds
	Note: By overly treating with Decolorizer solution, the dye will be washed away from Gram-positive bacteria as well.	
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. It is possible to use other Gram Decolorizer solutions from BioGnost's product range. If unstabilized iodine solution is used during the staining process, i.e. Gram Lugol solution, it is recommended to use Gram Decolorizer solution 1. If rapid destaining is necessary, then it is recommended to use Gram Decolorizer solution 3.

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 the BioGram 4 set in a tightly sealed original packaging at a 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. 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		Storage temperature range		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						