

BIOGRAM HISTO KIT

IVD *In vitro* diagnostic medical device



Five-reagent kit for the identification of bacteria according to Gram in histology sections For differentiation of Gram-positive from Gram-negative bacteria

INSTRUCTIONS FOR USE

REF Catalogue number: BGRH-100T (7x30 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. BioGnost's BioGram Histo kit contains Gram Crystal Violet 1% solution, stabilized Gram Lugol solution, two packages of Gram Decolorizer 2 solution, Gram Safranin solution and two packages of picric acid in acetone. Its characteristics make it an optimal bacteria staining agent which provides consistent results.

Product description:

- **BIOGRAM HISTO KIT** - Five-reagent kit in 7 packages for differentiating bacteria according to Gram in five steps

The kit contains:	100 tests (BGRH-100T)
Gram Crystal Violet 1% solution	30 mL (GC1-OT-30)
Gram Lugol solution, stabilized	30 mL (GLS-OT-30)
Gram Decolorizer 2 solution	2x30 mL (GD2-OT-30)
Gram Safranin solution	30 mL (GSF-OT-30)
Picric acid in acetone, solution	2x30 mL (PKA-OT-30)

Other sections and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

Preparing the histological sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 µm slices and place them on a VitroGnost glass slide.

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Stain with Gram Crystal Violet 1% solution	1 min
6.	Pour excessive dye off the section. Rinse the section carefully using stabilized Gram Lugol solution.	
7.	Fix the dye by treating the section using stabilized Gram Lugol solution	1 min
8.	Rinse the section carefully with distilled/demineralized water.	5 seconds
9.	Treat the preparation using Gram Decolorizer 2 solution. 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.	

10.	Rinse the section carefully with distilled/demineralized water.	5 seconds
12.	Treat the preparation using Gram Safranin solution.	1 min
13.	Rinse the section carefully with distilled/demineralized water.	5 seconds
14.	Treat the section with picric acid in acetone	5-10 seconds
15.	Dehydrate in 100% alcohol in two exchanges (Histanol 100)	2 exchanges, 10 short dips
16.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each
17.	Add a drop of immersion oil on the section (Cedar or Immersion oil).	
18.	Examine the section under immersion lens.	

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with VitroGnost cover glass.

Results

Gram-positive bacteria - blue-purple

Gram-negative bacteria - red

Background coloration - hues of purple-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 BioGram Histo kit 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. 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	 Product code	 European Conformity
 Refer to supplied instructions	 Keep away from heat and sunlight	 Valid until	 Lot number	 Manufacturer
 For <i>in vitro</i> diagnostic use only	 Keep in dry place	 Caution - fragile		

 BIOGNOST Ltd.
Medjugorska 59
10040 Zagreb
CROATIA
www.biognost.com



