

# BIOGRAM HISTO KIT



IVD In vitro diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - Class A device

## Five-reagent kit for identification of bacteria acc. to Gram in histological samples For differentiation of Gram-positive from Gram-negative bacteria

### INSTRUCTIONS FOR USE

<b>BASIC UDI number</b>	385889212HPC4080299MCKA		
<b>EMDN code</b>	W0104080299		
<b>REF</b>	<b>Catalog number</b>	<b>Volume</b>	<b>UDI-DI number</b>
BGRH-100T	Za 100 testiranja		03858890007466



### Intended use and test principle

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 for differentiating bacteria according to Gram

The kit contains:	100 tests (BGRH-100T)	Storage temperature
Gram Crystal Violet 1% solution	30 mL (GC1-OT-30)	15 – 25°C
Gram Lugol solution, stabilized	30 mL (GLS-OT-30)	15 – 25°C
Gram Decolorizer solution 2	2x30 mL (GD2-OT-30)	15 – 25°C
Gram Safranin solution	30 mL (GSF-OT-30)	15 – 25°C
Picric acid in acetone, solution	2x30 mL (PKA-OT-30)	15 – 25°C

### Additional reagents and materials that can be used in this method

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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New  
Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue  
Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua  
VitrGnost slides and coverslips for use in histopathology and cytology  
BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade

### Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 µm thin slices and mount on a VitrGnost microscope slide

### NOTE

Apply the reagent so it completely covers the section.

### Sample staining procedure

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Stain with Gram Crystal Violet 1% solution (≥5 drops)	1 min
6.	Pour excessive dye off the slide	
	Carefully rinse the slide using stabilized Gram Lugol solution	
7.	Fix the dye by treating the section using stabilized Gram Lugol solution (≥5 drops)	1 min
8.	Rinse the slide carefully with distilled/demineralized water	5 sec
9.	Treat the slide using Gram Decolorizer solution 2 (≥5 drops) End the process when the section turns grey-blue	10-15 sec
	Note: By overly treating with Decolorizer solution, the dye will be washed away from Gram-positive bacteria as well	
10.	Rinse the slide carefully with distilled/demineralized water	5 sec
12.	Treat the slide using Gram Safranin solution (add ≥ 5 drops)	1 min
13.	Rinse the slide carefully with distilled/demineralized water	5 sec
14.	Treat with Picric acid in acetone (≥5 drops)	10-30 sec
	Note: Treat until background pink coloration is washed away.	
15.	Dehydrate in 100% alcohol (Histanol 100)	2 exchanges of 10 short dips

16.	Clear in xylene (BioClear) or in a xylene substitute (BioClear New)*	2 exchanges, 2 min each
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Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the slide. 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.

\* To avoid discoloration (yellow washing away) it is advised to clear in xylene and mount on cover glass using BioMount DPX or BioMount DPX New.

### Result

Gram-positive bacteria – blue-purple

Gram-negative bacteria – red

Background coloration – pale yellow

### Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

### Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control.

If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

### Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

### Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product label.

### References

- Carson, F. L., Hladik, C. (2009): *Histotechnology: A Self-Instructional Text*, 3<sup>rd</sup> ed., Chicago: ASCP Press
- Kiernan, J. A. (2008): *Histological and Histochemical Methods*, 4<sup>th</sup> ed., Bloxham: Scion Publishing Ltd

Warnings and precautions regarding the materials contained in the product:	
	H225 Highly flammable liquid and vapor. H226 Flammable liquid and vapor. H319 Causes serious eye irritation H336 May cause drowsiness or dizziness. H241 Use explosion-proof electrical/ventilating/light equipment. EUH066 Repeated exposure may cause skin dryness or cracking.
	P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking P233 Keep container tightly closed. P273 Avoid release to the environment. P280 Wear protective gloves/protective clothing/eye protection/face protection. P281 Use personal protective equipment as required. P305+P351+P338 IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. P303+P361+P353 IF ON SKIN (or hair): take off immediately all contaminated clothing. Rinse skin with water (or shower). P337+P313 If eye irritation persists: get medical advice/attention.

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 Manufacturer	 Batch code	 Temperature limit	 <i>In vitro</i> diagnostic medical device	 Unique device identifier
 Date of manufacture	 Catalogue number	 Consult Instructions for use	 Contains sufficient for <n> tests	
 Use-by date	 Fragile, handle with care	 Caution	 European conformity	

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Version	Description / reason for change	Date
5	Revised in acc. to Regulation (EU) 2017/746 - IVDR	02.02.2026.