

# BIOGRAM 4 KIT

CE IVD *In vitro* diagnostic medical device

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

## Four-reagent kit for identification of bacteria acc. to Gram 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>
BGR4-100T	100 testiranja	03858890002843
BGR4-K-100	5x100 mL	03858888822682
BGR4-K-250	5x250 mL	03858888822699
BGR4-K-500	5x500 mL	03858888822705



#### 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. 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:	for 100 tests (BGR4-100T)	5x100 mL (BGR4-K-100)	5x250 mL (BGR4-K-250)	5x500 mL (BGR4-K-500)	Storage temperature
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)	15-25 °C
Gram Lugol solution, stabilized	30 mL (GLS-OT-30)	100 mL (GLS-OT-100)	250 mL (GLS-OT-250)	500 mL (GLS-OT-500)	15-25 °C
Gram Decolorizer solution 2	2x30 mL (GD2-OT-30)	2x100 mL (GD2-OT-100)	2x250 mL (GD2-OT-250)	2x500 mL (GD2-OT-500)	15-25 °C
Gram Safranin solution	30 mL (GSF-OT-30)	100 mL (GSF-OT-100)	250 mL (GSF-OT-250)	500 mL (GSF-OT-500)	15-25 °C

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

- VitroGnost 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

#### 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.

#### NOTE

Apply the reagent so it completely covers the slide

#### Sample staining procedure

##### a) using the kit for 100 tests (BGR4-100T)

1.	Stain with Gram Crystal Violet 1% solution (≥5 drops)	1 min
2.	Pour excessive dye off the slide	
3.	Rinse the slide carefully using Gram Lugol solution, stabilized	
4.	Fix the dye by treating the slide using stabilized Gram Lugol solution (≥5 drops)	1 min
5.	Rinse the slide carefully with distilled/demineralized water	5 sec
6.	Treat the preparation using Gram Decolorizer solution 2 (≥5 drops) End the process when the slide 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	
7.	Rinse the slide carefully with distilled/demineralized water	5 sec
8.	Stain the slide using Gram Safranin solution (≥5 drops)	1 min
9.	Rinse the slide carefully with distilled/demineralized water	5 sec
10.	Dry the slide using filter paper or let it dry by air	
11.	Add a drop of immersion oil on the slide (Cedar or Immersion oil).	
12.	Examine the slide under immersion lens	

**b) using the four-reagent kit of 100, 250 and 500 mL (BGR4-K-100, BGR4-K-250 i BGR4-K-500)**

Pour the reagents into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

1.	Stain with Gram Crystal Violet 1% solution	1 min
2.	Pour excessive dye off the slide	
3.	Rinse the slide carefully using Gram Lugol solution, stabilized.	
4.	Fix the dye by treating the slide using stabilized Gram Lugol solution	1 min
5.	Rinse the slide carefully with distilled/demineralized water	5 sec
6.	Treat the preparation using Gram Decolorizer solution 2 End the process when the slide 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	
7.	Rinse the slide carefully with distilled/demineralized water	5 sec
8.	Treat the preparation using Gram Safranin solution	1 min
9.	Rinse the slide carefully with distilled/demineralized water	5 sec
10.	Dry the slide using filter paper or let it dry by air	
11.	Add a drop of immersion oil on the slide (Cedar or Immersion oil)	
12.	Examine the slide under immersion lens	

**Result**

Gram-positive bacteria – blue-purple

Gram-negative bacteria – red

**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, mounting of the slides, 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**

1. Carson, F. L., Hladik, C. (2009): *Histotechnology: A Self-Instructional Text*, 3<sup>rd</sup> ed., Chicago: ASCP Press
2. 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:	
	<p>H225 Highly flammable liquid and vapor.                      H226 Flammable liquid and vapor.                      H319 Causes serious eye irritation.                      H336 May cause drowsiness or dizziness.                      H412 Harmful to aquatic life with long-lasting effects.                      EUH066 Repeated exposure may cause skin dryness or cracking.</p> <p>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.                      P305+P351+P338 IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.</p>

BGR4-IFU\_ENV10, 18.02.2026. IŠP

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

 **BioGnost Ltd.**  
 Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

Version	Description / reason for change	Date
10	Revised in acc. to Regulation (EU) 2017/746 - IVDR	18.02.2026.