BIOGNOST®

GIEMSA HP KIT

IVD *In vitro* diagnostic medical device CE

Kit for staining *Helicobacter pylori* in histology sections according to Lennart

INSTRUCTIONS FOR USE

REF Catalogue number: GMHP-100T (for 100 tests)

Introduction

Polychromatic Romanowsky dyes are a standard in hematology of blood smears and bone marrow. Giemsa is one of the Romanowsky dyes and beside hematology, it can be used in histology for visualization of *Helicobacter pylori* in gastroscopic samples of stomach. This staining method is also known as slow Giemsa staining according to Lennart. Giemsa solution may also be used to identify blood parasites, inclusion bodies and mastocytes in histology sections. Giemsa HP kit for 100 tests contains reagents for differentiation and rehydration in Ready-to-Use jars, enabling the sections to be directly immersed during the staining procedure. The jars are closed after the use, and reagents can be reused.

Product description

- **GIEMSA HP KIT**- Four-reagent kit for staining *Helicobacter pylori.*

The kit contains:	for 100 tests (GMHP-100T)
Giemsa solution	50 mL (GM-OT-50)
Buffer solution, pH 7.2	100 mL (PUF72-OT-100)
Differentiating reagent for Giemsa HP kit	2 x 100 mL (RGHP-OT-100)
Dehydrating reagent	2 x 100 mL (RDH-OT-100)
Plastic graded Pasteur pipette	1 pc

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

Preparing the histological sections for staining

- Fixate 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.

Sample staining procedure

During using Glemsa HP kit for 100 tests (product code GMHP-100T) immerse the sections directly in Ready-to-Use jars containing reagents for differentiation and dehydration. Filter the reagents if necessary.

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.	Preparation of **working Giemsa solution:** mix 43 ml of distilled (demi) water and 5 ml of Buffer solution, pH 7.2. Add 2 ml of Giemsa solution and mix.	
6.	Immerse the sections into the working Giemsa solution	30-60 minutes
	Note: discard Giemsa working solution after staining	
7.	Rinse in distilled (demi) water	
8.	Differentiate in differentiating reagent (part of Giemsa HP kit)	several rapid dips
	Note: if the section is treated with Differentiating reagent for too long, it may result in excessive release of blue-purple dye off the section	
9.	Dehydrate the section in Dehydrating reagent	1-2 minutes
	Note: if the section is treated with Dehydrating reagent for too long, it may result in excessive release of blue-purple dye off the section	
10.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

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 a VitroGnost cover glass.

Result

Helicobacter pylori - dark blue
Nuclei - blue
Cytoplasm - pink to blue

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

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 handling. 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.

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. Reagents 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 Giemsa HP kit in a tightly closed original package at temperature between 15°C and 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. Beck, R.C. (1938): *Laboratory Manual of Heamtological Technique,* Philadelphia, W.B. Saunders & Co.
2. Dacie, J. et Lewis S. (1995): *Practical haematology*, 4th ed., London, Churchill Livingstone.
3. Giemsa, G. (1922): Das Wesen der Giemsa-Farbung, *Zentralb f Bakt*; 89, 99-106.
4. International Commitee for Standardization in Haematology (1984): ICSH reference method for staining of blood and bone marrow films by azure B and eosin Y (Romanowsky stain), *British Journal of Haematology,* 57, p 707-710.
5. May, R. et Grünwald L. (1909): *Über die Farbung von Feutchpraparaten mit meiner Azur-Eosine methode,* Deutsche med Xschr, 35, pp 1751-1752.

GMHP-X, V5-EN3, 25 February 2019, AK/VR

	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	CE
	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						