BIOGNOST®

HEMATOXYLIN HP, PAP 1A

CE IVD *In vitro* diagnostic medical device Classification according to Regulation (EU) 2017/746 – **Class A** product

Modified Harris hematoxylin for nuclear staining acc. to Papanicolaou

High-intensity reagent for progressive and regressive staining in exfoliative cytology

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC30708STARVF				
EMDN code	W01030708				
REF **Catalog number**	**Volume**	**UDI-DI number**	REF **Catalog number**	**Volume**	**UDI-DI**
HEMHP-OT-100	100 mL	03858888824624	HEMHP-OT-2.5L	2500 mL	03858888824631
HEMHP-OT-500	500 mL	03858888821838	HEMHP-OT-5L	5000 mL	03858892120439
HEMHP-OT-1L	1000 mL	03858888820336			

Intended use and test principle

BioGnost's Hematoxylin HP, Pap 1A is one of the hematoxylin formulations used in cytology for precise staining of cell nuclei. Unlike Hematoxylin H, which is used in histology, Hematoxylin HP, Pap 1A is ideal for intensive staining of cytological smears by both the progressive and regressive methods. Hematoxylin is obtained by extraction from logwood (*Haematoxylon campechianum* L.). By oxidizing hematoxylin into hematein and binding it with metal ions (mordants), hematein becomes an indispensable nuclear stain. The positively charged hematein–mordant complex binds to the negatively charged phosphate ions of nuclear DNA, producing the characteristic blue staining. BioGnost's Hematoxylin HP, Pap 1A is a specific hematoxylin solution used for staining the chromatin of normal and abnormal cytological smears. It yields excellent staining results for the nuclear membrane, nucleoplasm, and nucleolus. Samples for testing can be gynecological and non-gynecological, such as sputum, urine, and samples obtained by cytological puncture. In order to obtain optimal staining results, BioGnost's Hematoxylin HP, Pap 1A reagent is fully compatible in its characteristics with BioGnost's other reagents for Papanicolaou cytological staining – OG-6, Pap 2A reagent, EA 31, Pap 3A reagent, as well as alternative counterstain polychromatic dyes such as EA 50, Pap 3B reagent, EA 65, Pap 3C reagent, and EA 65, Pap 3D reagent.

Product description

- **HEMATOXYLIN HP, PAP 1A** – Reagent for progressive and regressive nuclear staining in cytology. Contains optimally oxidized hematoxylin (hematein), aluminum ions, stabilizers, and antioxidants

Example of use of HEMATOXYLIN HP, PAP 1A

Additional reagents and materials that can be used in the staining method

- A fixative such as BioGnost's Cytospray solution
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX New, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- BioGnost reagents for Pap staining: EA 31 reagent, Pap 3A (or EA 50 reagent, Pap 3B) and OG-6, Pap 2A reagent

Preparation of cytological smear for staining

There are two methods for collecting and preparing cytological samples:

1. After collecting the cytological sample by swab, apply it to a slide (VitroGnost), immediately fix it with the fixative from the spray bottle (CitoSpray), dry, and store until the staining procedure. The cytological sample can also be fixed and stored until staining by immersion in a 95% alcohol solution (Histanol 95) for a minimum of 30 minutes.
2. Using the liquid-based cytology method (LBC, Liquid-Based Cytology) with a brush for collecting cytological samples, immediately fix the sample (CitoFix, CitoFix in transport containers) by detaching the brush head and immersing it in the fixative. At the beginning of cytological sample processing, separate the cells from the fixation fluid (one method is to centrifuge the fixation fluid) and apply them to a slide so that the cells are evenly distributed in a single layer. The cytological sample prepared in this way is ready for staining.

Staining procedure for cytological samples according to the Papanicolaou method

The start of the staining process depends on the way in which the cytological sample was collected and fixed to the microscopy slide.
If the sample is dry and previously fixed with CitoSpray, it should be kept in 95% alcohol (Histanol 95) for 10 minutes before staining to remove polyglycol. If the preparation is fixed with a 95% alcohol solution (Histanol 95), this step is unnecessary. During the staining procedure for cytological samples prepared by the liquid-based cytology method (LBC) containing a low concentration of alcohol, rehydration with a descending series of alcohol solutions is not necessary. The procedure begins with rinsing the preparation with distilled (demi) water, and continues with the staining process using Hematoxylin HP, Pap 1A.

NOTE

Apply the reagent so that it completely covers the preparation.

A) progressive staining method

1.	Rehydrate in a descending series of alcohols (Histanol 95 and Histanol 70) and in distilled/demineralized water	3 exchanges of 10 dips
2.	Stain with Hematoxylin HP, Pap 1A reagent	30 s
	Note: Prolonged exposure of the preparation to Hematoxylin HP, Pap 1A reagent may stain the cytoplasm in addition to the nucleus.	
3.	Rinse in distilled/demineralized or tap water	30 s
4.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: In the absence of the aforementioned reagents, rinse the preparation under an indirect stream of running water	3–5 min
5.	Dehydrate in an ascending series of alcohols (Histanol 70 and Histanol 95)	2 exchanges of 10 dips
6.	Stain with OG-6, Pap 2A reagent	2 minutes
7.	Rinse in 95% alcohol through two changes (Histanol 95)	2 exchanges, 30 s each
8.	Stain with EA 31 reagent, Pap 3A or EA 50 reagent, Pap 3B	4 minutes
9.	Rinse and dehydrate in 95% alcohol through two changes (Histanol 95)	2 exchanges, 1 min each
10.	Dehydrate in 100% alcohol through two changes (Histanol 100)	2 exchanges, 1 min each
11.	Clear in xylene (BioClear) or xylene substitute through two exchanges (BioClear New)	2 exchanges, 2 min each

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

B) regressive staining method

The regressive staining method generally achieves better differentiation of the sample and clearer visibility of nuclear structures

1.	Rehydrate in a descending series of alcohols (Histanol 95 and Histanol 70) and in distilled/demineralized water	3 exchanges of 10 dips
2.	Stain with Hematoxylin HP, Pap 1A reagent	4 min
3.	Rinse in distilled/demineralized or tap water	30 s
4.	Differentiate with HCL Pap reagent or in 0.1% HCl solution	5–10 s
	Note: This step removes excess hematoxylin from the nucleus and cytoplasm. If the preparation has been treated with the differentiating agent for too long, the nuclei may become destained.	
5.	Rinse in distilled/demineralized or tap water	10 dips
6.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: In the absence of the aforementioned reagents, rinse the preparation under an indirect stream of running water.	3–5 min
7.	Dehydrate in an ascending series of alcohols (Histanol 70 and Histanol 95)	2 exchanges of 10 dips
8.	Stain with OG-6, Pap 2A reagent	2 min
9.	Rinse in 95% alcohol through two changes (Histanol 95)	2 exchanges, 30 s each
10.	Stain with EA 31 reagent, Pap 3A or EA 50 reagent, Pap 3B	4 min
11.	Rinse and dehydration in 95% alcohol through two changes (Histanol 95)	2 exchanges, 1 min each
12.	Dehydrate in 100% alcohol through two changes (Histanol 100)	2 exchanges, 1 min each
13.	Clear in xylene (BioClear) or xylene substitute through two exchanges (BioClear New)	2 exchanges, 2 min each

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

Note

If precipitation has occurred in the Hematoxylin HP, Pap 1A solution or if a metallic sheen has formed on the surface, the reagent must be filtered before use.

Result

Nuclei – blue
Keratinized cells – yellow-orange
Superficial epithelial squamous cells, erythrocytes, nucleoli, cilia – pink-red
Cytoplasm of all other cell types (parabasal and intermediate squamous cells, columnar cells, polymorphonuclear leukocytes, lymphocytes, histiocytes, adenocarcinoma cells, undifferentiated carcinoma cells) – green

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in BioGnost's instructions for use may cause variations in the 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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, staining and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards.
If a serious incident occurs during use or as a result of its use, please report it to the manufacturer and/or authorized representative and the 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, therefore it is necessary to implement the necessary human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use, 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 in 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 production date and expiration date are printed on the product label.

References

1. Gill, G.W., Frost, J.K, Miller, K.A. (1974): A new formula for half-oxidized hematoxylin formula that neither overstains nor requires differentiation. *Acta Cytol.* 1974;18:300-301.
2. Gill, G.W. (2006): Enviro-Pap: an environmental friendly, economical, and effective Pap stain. *Lab. Med.* 37: p. 105-108.
3. Harris, H.F. (1900): On the rapid conversion of haematoxylin into haematein in staining reactions. J. *Appl. Microsc.* 3: p. 777-780
4. Papanicolaou, G.N. (1954): A new procedure for staining vaginal smears. *Science.* 95: p. 438-439.

Warnings and precautions regarding the materials contained in the product:		
	H318	Causes serious eye damage.
	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 presentand easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
	P310	Immediately call a POISON CENTER/doctor.

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	Manufacturer	LOT	Batch code		Consult instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
14	Revised acc. to Regulation (EU) 2017/746 - IVDR	09.04.2026.