BIOGNOST®

HEMATOXYLIN HP, PAP 1A

IVD *In vitro* diagnostic medical device CE

Modified hematoxylin acc. to Harris for nuclear staining acc. to Papanicolaou

Strong intensity reagent for progressive and regressive staining in exfoliative cytology

INSTRUCTIONS FOR USE

REF Catalogue number: HEMHP-OT-100 (100 ml) HEMHP-OT-500 (500 ml) HEMHP-OT-1000 (1000 ml) HEMHP-OT-2.5L (2500 mL)

Introduction

BioGnost's Hematoxylin HP, Pap 1A is one of formulations of hematoxylin used in cytology for a more precise nuclear cell staining. Unlike Hematoxylin H which is used in histology, Hematoxylin HP, Pap 1A is ideal for intensive staining cytology smears using progressive and regressive methods. Hematoxylin is extracted from logwood (*Haematoxylon campechianum L.*). Hematoxylin oxidates to hematein and binds with metal ions (mordants), hematein turns into irreplaceable nuclear dye. Positively charged hematein-mordant complex then binds with negatively charged phosphate ions of the DNA's nucleus, creating characteristic blue coloration. BioGnost's Hematoxylin HP, Pap 1A is a specific hematoxylin solution used for staining chromatins of both normal and abnormal cytology smears. They stain nuclear membrane, nucleoplasm and nucleolus exceptionally well. Test samples can be gynecological and non-gynecological, such as sputum, urine, and cytological puncture samples. In order to obtain optimal staining results, BioGnost's Hematoxylin HP, Pap 1A properties are completely in accordance with other BioGnost's reagents used for cytological staining acc. to Papanicolaou - OG-6, Pap 2A reagent, EA 31, Pap 3A reagent, as well as alternative counterstain polychromatic stains, such as EA 50, Pap 3B reagent, EA 65, Pap 3C reagent, and EA65, Pap 3D reagent.

Product description

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

Preparing the cytological smear for staining

There are two methods of collecting and preparing the cytological samples:

1. After collecting the cytological sample, place it on the microscope slide (VitroGnost), fixate it immediately with a fixative in a spray bottle (CitoSpray), dry it and keep until the staining process. Cytological sample may be fixated and kept until staining by immersing into 95% alcohol solution (Histanol 95) for a minimum of 30 minutes.
2. Using liquid-based cytology method (LBC) and brush for collecting cytological samples, fixate the sample immediately (CitoFix, CitoFix in transport containers) by removing the brush head and immersing it in the fixative. At the beginning of processing the sample, isolate the cells from the fixative (one of the methods is to centrifuge the fixative) and place them on the microscope slide equally in a single layer. Cytological sample prepared in such a way is ready for staining.

The Papanicolaou staining method, PROGRESSIVE

The first stage of staining procedure depends on the method the cytological sample was collected and fixated on the microscope slide.

If the sample is dry and previously fixed using CitoSpray, it is necessary to keep it in a 95% alcohol solution (Histanol 95) for 10 minutes in order to remove polyglycols. If the section was fixated with a 95% alcohol solution (Histanol 95), ignore this step. During staining cytology samples (prepared by using the liquid based cytology method (LBC)) that contain low concentration of alcohol, rehydration by descending series of alcohol solutions is not necessary. The procedure starts by rinsing the section using distilled (demi) water and is then stained using Hematoxylin HP, Pap 1A reagent.

1.	Rehydrate in descending series of alcohols (Histanol 95, Histanol 70) and in distilled (demi) water	10 dips in each of the 3 exchanges
2.	Staining using Hematoxylin HP, Pap 1A reagent	30 seconds or 2-3 min
	Note: Longer exposure of the section to Hematoxylin HP Pap 1A reagent may also stain cyoplasm (apart from nucleus)	
3.	Rinse the section with tap or distilled water	30 seconds
4.	Blue using Scott's solution or Bluing reagent	1 min
5.	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 minutes
6.	Dehydrate in ascending series of alcohols (Histanol 70 and Histanol 95)	10 dips in each of the 2 exchanges
7.	Stain using OG-6, Pap 2A reagent	2 min
8.	Rinse using 95% alcohol in two exchanges (Histanol 95)	30 seconds during each of the 2 exchanges
9.	Stain using EA 31, Pap 3A reagent or EA 50, Pap 3B reagent	4 min
10.	Rinse using 95% alcohol in two exchanges (Histanol 95)	1 minutes in each of the 2 exchanges
11.	Dehydrate in 100% alcohol in two exchanges (Histanol 100)	1 minutes in each of the 2 exchanges
12.	Clear the section in xylene (BioClear) or in xylene substitute (BioClear New) in two exchanges	2 minutes in each of the 2 exchanges

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.

Papanicolaou staining method, REGRESSIVE

The regressive staining method creates a better sample differentiation and clearer nuclear structure visibility.

The first stage of staining procedure depends on the method the cytological sample was collected and fixated on the microscope slide.

If the sample is dry and previously fixed using CitoSpray, it is necessary to keep it in a 95% alcohol solution (Histanol 95) for 10 minutes in order to remove polyglycols. If the section was fixated with a 95% alcohol solution (Histanol 95), ignore this step. During staining cytology samples (prepared by using the liquid based cytology method (LBC)) that contain low concentration of alcohol, rehydration by descending series of alcohol solutions is not necessary. The procedure starts by rinsing the section using distilled (demi) water and is then stained using Hematoxylin HP, Pap 1A reagent.

1.	Rehydrate in descending series of alcohols (Histanol 95, Histanol 70) and in distilled (demi) water	10 dips in each of the 3 exchanges
2.	Staining using Hematoxylin HP, Pap 1A reagent	4 min
3.	Rinse the section with tap or distilled water	30 seconds
4.	Differentiation using HCL Pap reagent or in 0.1% HCl solution	5-10 seconds
	Note: This step removes excessive hematoxylin from the nucleus and cytoplasm. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long.	
5.	Rinse the section with tap or distilled water	10 dips
6.	Blue using Scott's solution or Bluing reagent	1 min
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 minutes
7.	Dehydrate in ascending series of alcohols (Histanol 70 and Histanol 95)	10 dips in each of the 2 exchanges
8.	Stain using OG-6, Pap 2A reagent	2 min
9.	Rinse using 95% alcohol in two exchanges (Histanol 95)	30 seconds during each of the 2 exchanges
10.	Stain using EA 31, Pap 3A reagent or EA 50, Pap 3B reagent	4 min
11.	Rinse using 95% alcohol in two exchanges (Histanol 95)	1 minutes in each of the 2 exchanges
12.	Dehydrate in 100% alcohol in two exchanges (Histanol 100)	1 minutes in each of the 2 exchanges
13.	Clear the section in xylene (BioClear) or in xylene substitute (BioClear New) in two exchanges	2 minutes in each of the 2 exchanges

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.

Note

In the case of subsidence in the Hematoxylin HP, Pap 1A solution or formation of metallic glow on the surface, reagent should be filtered before use. Time periods of staining procedures are not completely standardized. The suggested methods are in accordance with BioGnost's reagents' properties and correspond to longtime clinical and laboratory practice. Intensity of staining depends on the period of exposure to stains and reagents. Staining procedure can be changed according to personal preferences if they correspond to the basic principles of cytotechnology.

Results

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

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. 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 Hematoxylin HP, Pap 1A in a tightly closed original package at temperature between +15°C and +25°C. Keep in dry 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. 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.* p37 105-108.
3. Harris, H.F. (1900): On the rapid conversion of haematoxylin into haematein in staining reactions. J. *Appl. Microsc.* p3 777-780
4. Papanicolaou, G.N. (1954): A new procedure for staining vaginal smears. *Science.* p95 438-439.

HEMHP-OT-X, V29-EN12, 12 July 2019, AK/IŠP

	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						