BIOGNOST®

HEMATOXYLIN G2

IVD *In vitro* diagnostic medical device CE

Hematoxylin acc. to Gill for nuclear staining
Moderate intensity new generation reagent for progressive staining in histopathology, cytology and counterstaining in immunohistochemistry

INSTRUCTIONS FOR USE

REF Catalogue number: HEMG2-OT-100 (100 mL) HEMG2-OT-500 (500 mL) HEMG2-OT-1L (1000 mL) HEMG2-OT-2.5L (2500 mL)

Introduction

BioGnost's Hematoxylin G2 is a high stability reagent and one of formulations of hematoxylin used in histopathology and cytology for a more precise nuclear cell staining. Compared to Hematoxylin G1, Hematoxylin G2 stains preparations with greater intensity due to double amount of hematoxylin dye. That results in shorter waiting periods. Hematoxylin G2 is ideal for darker, more intense staining of cellular nuclei of cytological smears or histological preparations, although it is also often used for contrast staining in immunohistochemistry. Unlike other hematoxylin formulations, hematoxylin acc. to Gill dyes goblet cells in the small intestine epithelium and the respiratory epithelium of the respiratory tract. 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. Hematoxylin acc. to GIll is a specific hematoxylin solution used for staining goblet cells and chromatins of both normal and abnormal tissue samples or cytological smears. BioGnost's Hematoxylin G1, G2 and G3 are half-oxidized, stabilized with glycols and contain aluminum ions. They stain nuclear membrane, nucleoplasm and nucleolus exceptionally well.

Product description:

- **HEMATOXYLIN G2** - Reagent used for progressive nuclear staining in histology, cytology and contrast staining in immunohistochemistry. It contains optimally oxidized hematoxylin, glycolic stabilizers and antioxidants.

Other sections and reagents that may be used with the procedure:

- 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 52/54, BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro.
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of BioGnost's glass slides
- Differentiation agent, such as BioGnost's Acid alcohol
- Bluing agents, such as BioGnost's Scott's solution or Bluing reagent
- 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 C, BioMount Aqua
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- Counterstaining reagents, such as BioGnost's eosin solutions
- Monochromatic reagent for cytoplasmic staining using the Papanicolaou method, such as BioGnost's OG-6 reagent, Pap 2A
- Polychromatic reagents for cytoplasmic staining using the Papanicolaou method, such as BioGnost's: EA 31 reagent, Pap 3A and EA 50 reagent, Papa 3B

A) Preparing histological sections for staining

- Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with an intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax 52/54, 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.

Hematoxylin and eosin (HE) staining procedure, progressive

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.	Stain using Hematoxylin G2	3-5 minutes
	Note: In the case of subsidence in the solution or a formation of metallic glow on the surface, reagent should be filtrated before use.	
6.	Immerse the section in distilled or demineralized water until dye is no longer being released from the section	
7.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: Finish the process of bluing after the nuclei turn blue If no Scott's solution or Bluing reagent is available, rinse the sections under tap water for 3-5 minutes.	
8.	Immerse the sections in distilled/demineralized water.	
9.	If alcoholic eosin solution is used, immerse the sections in 95% alcohol (Histanol 95). Skip this step if aqueous eosin solution is used.	
10.	Stain with one of eosin contrast solutions until the section is optimally stained	15 seconds - 2 minutes
	Note: Staining the sections in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposition time for eosin aqueous solutions is 90 seconds to 2 minutes	
11.	Rinse under tap water	2 min
12.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
13.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
14.	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 VitroGnost cover glass.

Results

Nuclei - blue
Cytoplasm, collagen, elastin, erythrocytes - various shades of pink (when staining with Eosin Contrast the shade is red-pink)
Goblet cells - dark blue

B) Preparing the cytological smear/sample or sample 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 G2.

1.	Rehydrate in descending series of alcohols (Histanol 95, Histanol 80 and Histanol 70) and in distilled or demineralized water	6-8 dips in each of the 4 exchanges
2.	Stain using Hematoxylin G2	2-5 minutes
3.	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
4.	Immerse the sections in distilled/demineralized water.	
5.	Dehydrate in ascending series of alcohols (Histanol 70, Histanol 80 and Histanol 95)	6-8 dips in each of the 3 exchanges
6.	Stain using OG-6 reagent, Pap 2A	2 minutes
7.	Rinsing using 95% alcohol in two exchanges (Histanol 95)	6-8 dips in each of the 2 exchanges
8.	Stain using EA 31 reagent, Pap 3A or EA 50 reagent, Pap 3B	4 minutes
9.	Rinse using 95% alcohol (Histanol 95)	6-8 dips
10.	Dehydrate using 100% alcohol (Histanol 100)	6-8 dips
11.	Dehydrate using 100% alcohol (Histanol 100)	3-5 minutes
12.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	6-8 dips
13.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3-5 minutes

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.

Results

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

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. 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 G2 in a tightly sealed original packaging at temperature of +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. Papanicolaou, G.N. (1954): A new procedure for staining vaginal smears. *Science.* p95 438-439.
4. Sheehan, D.C. et Hrapchak, B.B. (1980): *Theory and Practice of Histotechnology*, 2nd ed., St. Louise: CV Mosby Co.

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	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						