HEMATOXYLIN G2

IVD In vitro diagnostic medical device CE

Hematoxylin acc. to Gill for nuclear staining

High stability reagent used for progressive staining in histopathology, cytology and counterstaining in immunohistochemistry

INSTRUCTIONS FOR USE

REF Product code: HEMG2-OT-100 (100 mL) HEMG2-OT-110 (10x100 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. 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 cross section tissues 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, for instance BioNene on the limonene basis or BioClear New agent on the aliphatic hydrocarbons basis.
- Infiltration and fitting agent, such as BioGnost's granulated paraffin with polymers: BioWax Plus, 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
- 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 DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua, Canada Balsam or MountQuick Tube medium
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- Counterstaining reagents, such as BioGnost's eosin solutions: Eosin Y 0.2 aqueous, Eosin Y 0.5 aqueous, Eosin Y 1% aqueous, Eosin Y 2% aqueous, Eosin Y 0.5% alcoholic, Eosin Contrast
- Monochromatic reagent for cytoplasmic staining using the Papanicolaou method, such as BioGnost's OG-6 Pap reagent
- Polychromatic reagents for cytoplasmic staining using the Papanicolaou method, such as BioGnost's: EA 31 Papa reagent, EA 50 Papa reagent, EA 65 Papa reagent, EA 36 Papa reagent

A) Preparing the histological staining preparations

- 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 an intermedium; in xylene (BioClear) or in a xylene substitute (BioNene, 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

Hematoxylin and eosin (HE) staining procedure, progressive

- Deparaffinize the section using xylene (BioClear) or a xylene substitute (BioNene or BioClear New), then rehydrate the section through series of descending alcohol solutions (Histanol 100, Histanol 95, Histanol 80 and Histanol 70)
- Stain the section with Hematoxylin G2 solution by immersing it for in the solution 5 min or until an optimal level of staining is achieved
  Note: In the case of subsidence in the solution or a formation of metallic glow on the surface, reagent should be filtrated before use
- Rinse the section under running water for 3-5 min
- Stain the preparation with one of the contrasting solutions (Eosin Y 0.2 aqueous, Eosin Y 0.5 aqueous, Eosin Y 1% aqueous, Eosin Y 2% aqueous, Eosin Y 0.5% alcoholic, Eosin Contrast) by immersing it in the solution for 30 seconds to 2 min or until an optimal level of staining is achieved
  Note: If the alcohol solution of eosin is used, the section should be treated with a 95% alcohol solution (Histanol 95) by immersing it in the solution for 30 seconds.
  Staining the sections in Eosin Y 0.5% alcoholic and Eosin Contrast causes intensive eosinophil color to show much faster (in under 15 seconds time). Exposition time for Eosin Y 0.2% and Eosin 0.5% aqueous solutions is 2 min, for Eosin Y 1% aqueous is 90 seconds and for Eosin Y 2% aqueous 60 seconds.
- Immerse the section in water until it turns blue, that is, until the excessive amount of eosinophil color is washed off
- Dehydrate the section by immersing it into two exchanges of a 95% alcohol solution (Histanol 95). Repeat 10-15 times
- Completely dehydrate the section by immersing it into three exchanges of a 100% alcohol solution (Histanol 100). Repeat 10-15 times
- Clear the section by immersing it in two exchanges of xylene (BioClear) or a xylene substitute (BioNene, BioClear New). Repeat 10-15 times
- Apply suitable BioMount covering medium. If BioClear xylene was used, use one of the xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, Canada Balsam or MountQuick Tuba). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New
- Cover the section with a VitroGnost cover glass

Result

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

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) or immerse it in a 95% alcohol solution (Histanol 95) for at least 30 min.
   Note: The sample must not get dried before fixation!

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.
   Note: The staining procedure for cytological samples prepared using the method of liquid-based cytology begins from step 3, i. e. rinsing the section with distilled water.

The Papanicolaou staining method, <u>progressive</u>

Note: The staining procedure for cytological samples prepared using the method of liquid-based cytology begins from step 3, i. e. rinsing the section with distilled water.

- Treat the section with a 95% alcohol solution by immersing it in the solution for 10 min (Histanol 95)
  Note: After the sample was fixated with a fixative in a spray bottle (CitoSpray), the section should be washed with a 95% alcohol solution (Histanol 95) in order to remove polyglycols from the CitoSpray fixative. If the section was fixated with a 95% alcohol solution (Histanol 95), ignore this step.
- Rehydrate the section through series of descending alcohol solutions (Histanol 80) and a 70% (Histanol 70) alcohol solution. Repeat 6-8 times
- Immerse the section in distilled or demineralized water 6-8 times
- Stain the section with Hematoxylin G1 solution (acc. to Gill) by immersing it in the solution for 2-5 min or with Hematoxylin HP solution (acc. to Harris for cytology) by immersing it for 2-3 min in the solution or until an optimal level of staining is achieved
- Rinse the section under running water until dye is no longer being released from the preparation
- Treat the previously stained section with a bluing agent (Scott's solution, Bluing reagent)
  Note: End the process of bluing after the nuclei turn blue.
- Dehydrate the section through series of ascending alcohol solutions (Histanol 70, Histanol 80, and Histanol 95)
- Stain the section with OG-6 Papa monochromatic reagent by immersing it in the reagent for 2-3 min or until an optimal level of staining is achieved
- Treat the section by immersing it into two exchanges of a 95% alcohol solution (Histanol 95). Repeat 6-8 times
- Stain the section using a EA 31 or EA 50 Pap polychromatic reagent by immersing it in the reagent for 2-3 min or until an optimal level of staining is achieved.
  Note: In the case of subsidence in the solution, reagent should be filtrated before use.
- Treat the section by immersing it into three exchanges of a 95% alcohol solution (Histanol 95). Repeat 6-8 times
  Note: Contrasting dyes from the section could get washed off if the section stays in the alcohol solution for a longer period of time.
- Completely dehydrate the section by immersing it into three exchanges of a 100% alcohol solution (Histanol 100). Repeat 15-20 times
- Clear the section in xylene (BioClear) or in a xylene substitute (BioNene, BioClear New) by immersing it for 5 min
- Apply suitable BioMount covering medium. If BioClear xylene was used, use one of the xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, Canada Balsam or MountQuick Tuba). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New
- Cover the section with a VitroGnost cover glass

Result

Blue - nuclei
Yellow-range - keratinized cells
Pink-orange/red - superficial squamous epithelial cell, erythrocytes, nucleoli, cilia
Green - cytoplasm of all the other cell types (parabasal and intermediate squamous cells, columnar cells, polymorphonuclear leukocytes, lymphocytes, histiocytes, adenocarcinomas, undifferentiated carcinoma cells)

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.

HEMG2-OT-X, V8-EN8, 24 Jun 2016, IŠP/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						