HEMATOXYLIN G3

IVD In vitro diagnostic medical device CE

Hematoxylin acc. to Gill for nuclear staining
High stability reagent for regressive staining in histopathology and cytology

INSTRUCTIONS FOR USE

REF Product code: HEMG3-OT-100 (100 ml) HEMG3-OT-110 (10x100 ml) HEMG3-OT-500 (500 ml) HEMG3-OT-1L (1000 ml) HEMG3-OT-2.5L (2500 mL)

Introduction

BioGnost's Hematoxylin G3 is a high stability reagent and one of formulations of hematoxylin used in histopathology and cytology, as well as contrasting dye in immunohistochemistry for a more precise nuclear cell staining. Unlike other hematoxylin formulations, hematoxylin acc. to Gill dyes goblet cells in the small intestine epithelium and the respiratory epithelium of the respiratory tract. Compared to BioGnost's Hematoxylin G1 and G2, Hematoxylin G3 stains the sections with stronger intensity and time needed for getting the results of staining is shorter. 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 GIII is a specific hematoxylin solution used for staining goblet cells and chromatins of both normal and abnormal cross section tissues and 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 G3** - Reagent for regressive nuclear staining in histopathology and cytology. Contains optimally oxidized hematoxylin with sodium iodate, aluminum ions and antioxidants

Other slides 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, 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 56/58, BioWax Plus 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 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 Pap reagent, EA 50 Pap reagent, EA 65 Pap reagent, EA 36 Pap reagent

A) Preparing the histological staining preparations

- Fixate the tissue sample tightly (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 (BioNene, BioClear New)
- Infiltrate and fit the sample in paraffin (BioWax 56/58, BioWax Plus 56/68, 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, <u>regressive</u>

- 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) to distilled or demineralized water
- Stain the section with Hematoxylin G3 solution by immersing it in the solution for 8-15 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
- Immerse the section in distilled or demineralized water until dye is no longer being released from the section
- Differentiate the section by quickly immersing it 3-10 times in a differentiating agent (Acid alcohol).
  Note: This step removes excessive hematoxylin. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long
- Immerse the section briefly in distilled/demineralized water
- Immerse the section in a bluing agent (Scott's solution, Bluing reagent)
  Note: Finish the process of bluing after the nuclei turn blue
- 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 15 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
- Rinse the section under running water for 30 seconds
- Dehydrate the section by immersing it 10-15 times in two exchanges of a 95% alcohol solution (Histanol 95).
- Completely dehydrate the section by immersing it 10-15 times into three exchanges of a 100% alcohol solution (Histanol 100).
- Clear the section by immersing it 10-15 times in two exchanges of xylene (BioClear) or a xylene substitute (BioNene, BioClear New).
- 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 Tube). 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

B) Preparing the cytological smear/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 completely dry 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

Papanicolaou staining method, <u>regressive</u>

- 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 6-8 times in distilled or demineralized water
- Stain the section with Hematoxylin HP solution (modified acc. to Harris for cytology) by immersing it for 6 min or Hematoxylin G3 solution (acc. to Gill) by immersing it for 5 min or until an optimal level of staining is reached
  Note: In the case of subsidence in the solution or a formation of of metallic glow on the surface, reagent should be filtrated before use
- Immerse the section 6-8 times in distilled or demineralized water
- Differentiate the section by immersing it in the differentiating medium (0.1% HCl) for 5-10 seconds
  Note: This step removes excessive hematoxylin. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long
- Immerse the section 6-8 times in distilled or demineralized water
- Treat the section with a bluing agent (Scott's solution, Bluing reagent)
  Note: End the process of bluing after the nuclei turn blue
- Rinse the section under running water for 3-4 min (do not put the section under a direct stream of water)
- Dehydrate the section through series of ascending alcohol solutions (Histanol 70, Histanol 80, and Histanol 95). Repeat 6-8 times
- Stain the preparation with OG-6 Pap 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 6-8 times in two exchanges of a 95% alcohol solution (Histanol 95)
- Stain the section using an 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 6-8 times in two exchanges of a 95% alcohol solution (Histanol 95)
- Completely dehydrate the section with a 100% alcohol solution (Histanol 100) for 5 min
- Treat the section by immersing it 6-8 times in a blend of absolute alcohol (Histanol 100) and xylene (BioClear) or xylene substitute (BioClear New) in equal volumes
- Clear the section by immersing it 6-8 times in xylene (BioClear) or a xylene substitute (BioClear New)
- Clear the section in xylene (BioClear) or in a xylene substitute (BioNene, BioClear New) by immersing it for 2 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 Tube). 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-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 G3 in a tightly sealed original packaging 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. 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	Storage temperature range	Number of tests in package	Product code	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 number	Manufacturer		
For *in vitro* diagnostic use only	Keep in dry place	Caution - fragile				