BIOGNOST®

HEMATOXYLIN powder dye, C.I. 75290

IVD *In vitro* diagnostic medical device CE

Natural Black 1, BSC certified dye
For preparation nuclear staining reagents

INSTRUCTIONS FOR USE

REF Catalogue number: H-P-25 (25 g) H-P-100 (100 g)

Introduction:

Hematoxylin is extracted from logwood (*Haematoxylon campechianum L.*). Hematoxylin oxidates to hematein and binds with metal ions (mordants), hematein turns into irreplaceable nuclear color. Positively charged hematein-mordant complex then binds with negatively charged phosphate ions of the DNA's nucleus, creating characteristic blue coloration. There are various hematoxylin solutions, such as histological staining reagents (Hematoxylin H, Hematoxylin M, Hematoxylin ML, Hematoxylin G1, Hematoxylin G2, Hematoxylin G3, Hematoxylin PTA, Hematoxylin W kit) and cytology reagents (Hematoxylin G1, Hematoxylin G2, Hematoxylin G3, Hematoxylin HP). Although each of the listed reagents has a specific use, each provides excellent results of staining nuclear membrane, nucleoplasm and nucleolus.

Product description:

- **HEMATOXYLIN POWDER DYE** - Biological Stain Commission (BSC) certified powder dye for preparing the solution for nuclear staining

Other sections 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, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro.
- 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 DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- Counterstaining reagents, such as BioGnost's eosin solutions: Eosin aqueous 0.5%, Eosin aqueous 1.0%, Eosin 0.5% alcoholic, Eosin Contrast
- Potassium aluminum sulfate
- Sodium iodate
- Ethylene glycol
- Differentiation agent, such as BioGnost's Acid alcohol
- Bluing agents, such as BioGnost's Scott's solution or Bluing reagent
- Microscopy reagent, such as BioGnost's Acetic acid for histology

Preparation of the reagent:

- Hematoxylin acc. to Harris
  Dissolve 5 g of Hematoxylin powder dye in 50 ml of ethylene glycol (Histanol 100) while slowly heating. Heat 950 ml of distilled water and dissolve 100 g of potassium aluminum sulfate in it. Stir both solutions and heat gradually until boiling point. After the solution boils, remove from fire and add 370 mg of sodium iodate while constantly stirring. After it cools down, add 4 ml of BioGnost's Acetic acid for histology. Filter the reagent before use.

- Hematoxylin Gill 2
  Mix 250 g of ethylene glycol and 730 ml of distilled (demi) water. Add 4 g of hematoxylin, 0.4 g of sodium iodate and 70.4 g of aluminum sulfate octadecahydrate. Mix well and dissolve all the ingredients. Add 20 ml of Acetic acid for histology.

Preparing the histological sections for staining

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

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 with Hematoxylin (H, ML, G3)	4-8 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.	Differentiate using Acid alcohol	3-10 dips
	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.	
8.	Rinse in distilled water	
9.	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.	
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 a VitroGnost cover glass.

Result:

Nucleus - blue
Cytoplasm, collagen, muscle fibers, erythrocytes - shades of pink (red when staining with Eosin Contrast)

Note

Time periods of staining procedures are not standardized. 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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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 the Hematoxylin powdered dye in a tightly closed original package at temperature between 15°C and 25°C. Do not keep in cold places, do not freeze and avoid exposing to direct sunlight. Stability period is 12 months. Expiry date is printed on the product's label.

References

1. Baker, J.R. (1962): Experiments on the action of mordants. 2. Aluminium-hematein. *Q.J.Microsc. Sci.* p103 493-517.
2. Conn, J. (1977): *Biological Stains*, 9th ed., Baltimore: Williams and Wilkens Co.
3. Harris, H.F. (1898): A new method of “ripening” haematoxylin. *Microsc. Bull.* (Philadelphia) Dec. 47.
4. Harris, H.F. (1900): On the rapid conversion of haematoxylin into haematein in staining reactions. J. *Appl. Microsc.* p3 777-780.

H-X, V6-EN2, 14 February 2017, AK/VR

Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	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	Lot number	Manufacturer		
IVD For *in vitro* diagnostic use only	Keep in dry place	Caution - fragile					