

HEMATOXYLIN H



IVD *In vitro* diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - Class A device

Modified hematoxylin for nuclear staining acc. to Harris

Reagent for strong, regressive staining in histopathology

INSTRUCTIONS FOR USE

BASICI UDI number	385889212HPC30708STARVF				
EMDN code	W01030708				
[REF] Catalog number	Volume	UDI-DI number	[REF] Catalog number	Volume	UDI-DI number
HEMH-OT-100	100 mL	03858888823399	HEMH-OT-2.5L	2500 mL	03858890001259
HEMH-OT-500	500 mL	03858888823405	HEMH-OT-5L	5000 mL	03858892121603
HEMH-OT-1L	1000 mL	03858888820008	HEMH-OT-20L	20 L	03858890001860



Intended use and test principle

BioGnost's Hematoxylin H is one of the most well-known formulations of hematoxylin used in histopathology for a more precise nuclear cell staining. Hematoxylin acc. to Harris is applied using a progressive or a regressive method in a routine hematoxylin and eosin (HE) staining in histology. Hematoxylin is extracted from logwood (*Haematoxylon campechianum* L.). Hematoxylin oxidizes 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. The original Hematoxylin acc. to Harris formula is oxidized with mercury oxide. However, BioGnost's version of Hematoxylin acc. to Harris does not contain mercury oxide because of its toxicity; environment-friendly sodium iodate is used instead. Hematoxylin H stains nuclear membrane, nucleoplasm and nucleolus exceptionally well.

Product description

- **HEMATOXYLIN H** – Reagent for regressive nuclear staining in histopathology. Contains optimally oxidized hematoxylin with sodium iodate, aluminum ions and antioxidants

Additional reagents and materials that can 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 52/54, BioWax Plus 56/58, BioWax 56/68, BioWax Blue
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX New, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, types A, C, FF, 37 or Tropical Grade
- Reagents for nuclear staining such as BioGnost's eosin solutions
- Differentiation agent, such as BioGnost's Acid alcohol
- Nuclear bluing agents, such as BioGnost's Scott's solution or Bluing reagent

Preparing histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 µm thin slices and mount on a VitroGnost microscope slide

Hematoxylin and eosin (HE) manual* staining procedure, progressive

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 izmjene u trajanju od 5 i 3 minute
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Stain using Hematoxylin H	3-5 min
	If sedimentation occurs or a metallic sheen forms in Hematoxylin H solution, filter the reagent before use	
6.	Immerse the slide in distilled/demineralized water until dye is no longer being released from the slide	
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 slide under tap water for 3-5 minutes	
8.	Immerse slide in distilled/demineralized water	
9.	If alcoholic eosin solution is used, immerse the slide in 95% alcohol (Histanol 95). Skip this step if aqueous eosin solution is used	
10.	Stain with one of eosin counterstaining solutions until the section is optimally stained	15 sec to 2 min
	Note: Staining the slide in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended incubation time for eosin aqueous solutions is 90 seconds to 2 minutes	
11.	Rinse under tap water Note: If using an alcoholic eosin solution for counterstaining, skip this step	2 min
12.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
13.	Dehydrate in 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
14.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

* Automatic hematoxylin – eosin (HE) staining method is available in BioGnost's Hem Diff, Hem Diff Strong and BioBluing Buffer instructions for use.

Hematoxylin and eosin (HE) manual staining procedure, regressive

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min

3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Stain using Hematoxylin H	4-8 min
	If sedimentation occurs or a metallic sheen forms in Hematoxylin H solution, filter the reagent before use	
6.	Immerse the slide in distilled or demineralized water until dye is no longer being released from the slide	
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 slide is treated with the differentiation agent for too long	
8.	Rinse in distilled/demineralized 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 slide under tap water for 3-5 minutes	
10.	Immerse the slide in distilled/demineralized water	
11.	If alcoholic eosin solution is used, immerse the slide in 95% alcohol (Histanol 95). Skip this step if aqueous eosin solution is used	
12.	Stain with one of eosin counterstaining solutions until the slide is optimally stained	15 sec to 2 min
	Note: Staining the slide in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended incubation time for eosin aqueous solutions is 90 seconds to 2 minutes	
13.	Rinse under tap water Note: If using an alcoholic eosin solution for counterstaining, skip this step.	
14.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
15.	Dehydrate in 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
16.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

Result

Nucleus- blue

Cytoplasm, collagen, muscle fibers, erythrocytes – shades of pink

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in BioGnost's instructions for use may cause variations in the results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. It is necessary to follow the manufacturer's instructions for use. To avoid errors, staining of samples and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards. To avoid a false result, it is recommended to use a positive and negative control. If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use and in the safety data sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The production date and expiration date are printed on the product label.

Reference

1. Baker, J.R. (1962): Experiments on the action of mordants. 2. Aluminium-hematein. *Q.J.Microsc. Sci.* 103, str. 493-517.
2. Conn, J. (1977): *Biological Stains*, 9th ed., Baltimore: Williams and Wilkins 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.* 3: str. 777-780.

Warnings and precautions regarding the materials contained in the product:	
	H318 Causes serious eye damage.
	P280 Wear protective gloves/protective clothing/eye protection/face protection.
	P305+P351+P338 IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
	P310 Immediately call a POISON CENTER/doctor.

HEMH-IFU_ENV17, 16.01.2026., IŠP

	Manufacturer		Batch code		Consult instructions for use		European conformity
	Date of manufacture		Catalogue number		Caution		Unique device identifier
	Use-by date		Temperature limit		In vitro diagnostic medical device		

 **BioGnost Ltd.**
Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

Version	Description / reason for change	Date
17	Revised in acc to Regulation (EU) 2017/746 - IVDR	16.01.2026.