

HEMATOXYLIN, C.I. 75290

CE IVD In vitro diagnostic medical device

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

For preparation of nuclear staining reagents

Natural Black 1

INSTRUCTIONS FOR USE

Basic UDI-DI	385889212HPC30707PDYETD		
EMDN code	W01030707		
REF	Catalog number	Mass	UDI-DI
H-P-25		25 g	03858888820848
H-P-100		100 g	03858888820855



Intended use and test principle

Hematoxylin is obtained by extraction from logwood (*Haematoxylon campechianum L.*). By oxidizing hematoxylin to hematein and binding with metal ions (mordants), hematein becomes an indispensable nuclear stain. The positively charged hematein–mordant complex binds to the negatively charged phosphate ions of nuclear DNA, producing the characteristic blue staining. There are several different hematoxylin solutions, reagents for histological staining (Hematoxylin H, Hematoxylin M, Hematoxylin ML, Hematoxylin G1, Hematoxylin G2, Hematoxylin G3, Hematoxylin PTA, Hematoxylin W kit) and reagents for cytology (Hematoxylin G1, Hematoxylin G2, Hematoxylin G3, Hematoxylin HP). Although these reagents have their characteristic applications, each provides excellent staining results for the nuclear membrane, nucleoplasm, and nucleolus.

Product description

- **HEMATOXYLIN, C.I. 75290** – powder dye for the preparation of nuclear staining reagents

Example of using Hematoxylin powder dye for the preparation of Gill 2 hematoxylin in the progressive staining method

Additional reagents and materials that can be used in the method

- Fixatives, such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- A clearing agent such as BioClear xylene or a substitute such as BioClear New, an aliphatic hydrocarbon-based agent
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue
- Microscopic slide covering agents and cover glass mountants 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 slides and coverslips for use in histopathology and cytology
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- Counterstaining reagents such as BioGnost's eosin solutions: Eosin 0.5% aqueous, Eosin 1.0% aqueous, Eosin 0.5% alcoholic, Eosin Contrast
- A bluing agent such as BioGnost's Scott's solution or Bluing reagent
- Chemicals: glacial acetic acid (concentrated), sodium iodate, ethylene glycol, aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$)

Preparation of the staining solution

- Gill 2 hematoxylin (1000 mL):
Mix 750 mL of distilled/demineralized water with 250 mL of ethylene glycol. Add 4 g of Hematoxylin powder dye and stir until completely dissolved. Add 0.4 g of sodium iodate and stir until dissolved. Add 40 g of aluminum sulfate and stir for several minutes until the aluminum sulfate crystals are completely dissolved. Carefully add 20 mL of glacial acetic acid. Leave for approximately one hour at room temperature. Filter the reagent before use.

Preparation of 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 micron thin sections and mount on a VitroGnost microscope slide

Manual hematoxylin-eosin (HE) staining procedure, progressive

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydration in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydration in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Stain with Gill 2 hematoxylin	3-5 min
	Note: If precipitation has occurred in the solution or a metallic sheen has formed on the surface, the reagent must be filtered before use	
6.	Immerse the slide in distilled/demineralized water until the release of dye from the slide stops	
7.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: Stop bluing after the nuclei turn blue. If Scott's solution or Bluing reagent are unavailable, rinse the slides under running tap water for 3–5 minutes	
8.	Immerse the slide in distilled/demineralized water	
9.	If an alcoholic eosin solution is used, immerse the slides in 95% alcohol (Histanol 95). If an aqueous eosin solution is used, skip this step	within 2 min
10.	Stain with one of the eosin counterstain solutions until optimal staining of the preparation is achieved	15 s - 2 min
	Note: Staining slides with alcoholic eosin solutions produces an intense eosinophilic color much faster (within 15 seconds), whereas exposure of the preparation to aqueous eosin solutions is recommended for 90 seconds to 2 minutes	
11.	Rinse under running tap water	2 min
	Note: If an alcoholic eosin solution is used as counterstain, skip this step	

12.	Dehydration in 95% alcohol (Histanol 95)	2 exchanges of 10-15 dips
13.	Dehydration in 100% alcohol (Histanol 100)	3 exchanges of 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.

Result

Nuclei - blue

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

Note

The formulation described above is only one of the methods for preparing the staining solution. Depending on individual requirements and the laboratory's standard operating procedures, the staining solution may also be prepared according to other protocols.

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the method of preparing the dye solution or from the staining procedure may cause variations in the staining results shown in this Instructions for Use.

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 solution preparation, staining procedure and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards.

If a serious incident occurs during use or as a result of its use, please report it to the manufacturer and/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 and in BioGnost's 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.












References

1. Baker, J.R. (1962): Experiments on the action of mordants. 2. Aluminium-hematein. *Q.J. Microsc. Sci.* 103, pp. 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.* 3: pp. 777–780.

Warnings and precautions regarding the materials contained in the product:

	H319	Causes serious eye irritation.
	P280	Wear protective gloves/protective clothing/eye protection/face protection.
	P337+P313	If eye irritation persists: get medical advice/attention.
	P305+P351+P338	IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

H-P-IFU_ENV3, 13.04.2026., IŠP

 Proizvođač	 Broj serije	 Pročitati priloženu uputu	 Europska sukladnost
 Datum proizvodnje	 Kataloški broj	 Oprez	 Jedinstvena Identifikacija proizvoda
 Rok uporabe	 Temperaturno ograničenje	 Samo za <i>in vitro</i> dijagnostičku primjenu	

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Version	Description / reason for change	Date
3	Revised acc. to Regulation (EU) 2017/746 - IVDR	13.04.2026.