

HEMATOKSILIN M

CE IVD *In vitro* diagnostic medical device

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

Modified hematoxylin acc. to Mayer for nuclear staining

Reagent used for progressive staining in histopathology and counterstaining in immunohistochemistry

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF						
EMDN code	W01030708						
REF	Catalog number	Volume	UDI-DI number	REF	Catalog number	Volume	UDI-DI number
	HEMM-OT-100	100 mL	03858888822958		HEMM-OT-2.5L	2500 mL	03858890001242
	HEMM-OT-500	500 mL	03858888822965		HEMM-OT-20L	20000 mL	03858892121184
	HEMM-OT-1L	1000 mL	03858888820015				



Intended use and test principle

BioGnost's Hematoxylin M is one of the formulations of hematoxylin used in histopathology and immunohistochemistry for a more precise nuclear cell staining. It is applied progressively in a routine hematoxylin and eosin (HE) staining and counterstaining in immunohistochemistry. Unlike other hematoxylin formulations, modified hematoxylin acc. to Mayer does not contain alcohol, which makes it suitable for using in reactions with chromogenic aminoethylcarbazole (AEC), which is alcohol soluble. Hematoxylin is extracted from logwood (Haematoxylin 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. BioGnost's modified hematoxylin acc. to Mayer does not contain toxic antioxidants. Environment-friendly sodium iodate is used instead. Along with aluminum ions, it contains a low level of hematoxylin that selectively stains chromatin without staining the cytoplasm, which provides outstanding results in staining the cellular membrane, nucleoplasm and nucleolus.

Product description

- **HEMATOXYLIN M** - Reagent used for progressive nuclear staining in histopathology and counterstaining in immunohistochemistry. Contains optimally oxidized hematoxylin with sodium iodate, chloral hydrate stabilizer and antioxidants.

Additional reagents and materials that can be used in this method

- 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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue
- 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
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade
- Reagents for nuclear staining such as BioGnost's eosin solutions
- Nuclear bluing agents, such as poput BioGnost's Bluing reagent or Scott's solution

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 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 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 M	3-10 min
	If sedimentation occurs or a metallic sheen forms in Hematoxylin M, 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 in distilled/demineralized water	
9.	If using an alcoholic eosin solution immerse the samples into 95% alcohol (Histanol 95). If using an aqueous eosin solution, skip this step.	
10.	Stain using one of eosin contrast solutions until optimal staining is achieved	15 sec to 2 min
	Note: Staining the slides 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 contrast solution, 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 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 VitroGnost cover glass.
 * Hematoxylin and eosin (HE) automatic staining procedure is available Instructions for use for the following BioGnost products: Hem Diff, Hem Diff Strong and BioBluing buffer.

Result

Nuclei – blue

Cytoplasm - hues of pink (red – pink if staining with Eosin contrast)

Collagen, elastin, erythrocytes – orange – yellow (red – orange if staining with Eosin contrast)

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining 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. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards.

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, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label 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 expiration date is printed on the product label.

References

- Lillie, R.D. (1977): Conn's Biological Stains, 9th ed., Baltimore, Williams and Wilkens Co.
- Mayer, P. (1891): Über das Färben mit Hämatoxilin. Z. Wiss. Mikrosk. 8, str. 337-341.
- Mayer, P. (1904): Notiz über Hämatein und Hämalan, Y. Wiss. Mikrosk., 20, str. 409-4011.

Warnings and precautions regarding the materials contained in the product:	
	<p>H302 Harmful if swallowed.</p> <p>P280 Wear protective gloves/protective clothing/eye protection/face protection. P301 + P312 IF SWALLOWED: call POISON CENTER/doctor if you feel unwell.</p>

HEMM-IFU_HRV11, 23.02.2026., IŠP

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

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

Version	Description / reason for change	Date
11	Revised acc. to Regulation (EU) 2017/746 - IVDR	23.02.2026.