

HEMATOXYLIN M

IVD *In vitro* diagnostic medical device



Modified hematoxylin acc. to Mayer for nuclear staining

Reagent used for progressive staining in histopathology and counterstaining in immunohistochemistry

INSTRUCTIONS FOR USE

REF Catalogue number: HEMM-OT-100 (100 ml) HEMM-OT-500 (500 ml) HEMM-OT-1L (1000 ml) **HEMM-OT-2.5L (2500 mL)** HEMM-OT-20L (20 L)

Introduction

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.

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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax 52/54, BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro
- Glass slides used in histology, pathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR, or one of 30 (and more) BioGnost's glass slides
- 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
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- Counterstaining reagents, such as BioGnost's eosin solutions:

Preparing 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 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μ m slices and place them on a VitroGnost glass slide.

Hematoxylin and eosin (HE) staining procedure, progressive

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 the section with Hematoxylin M	3-10 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.	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.	
8.	Immerse the sections in distilled/demineralized water.	
9.	If alcoholic eosin solution is used, immerse the sections in 95% alcohol (Histanol 95). Skip this step if aqueous eosin solution is used.	
10.	Stain with one of eosin contrast solutions until the section is optimally stained	15 seconds to 2 min
	Note: Staining the sections in Eosin Y 0.5% alcoholic and Eosin Contrast causes intensive eosinophil color to show much faster (1 minute is sufficient). Exposition time for Eosin Y 0.5% and Eosin 1% aqueous is 2 min and 90 seconds, respectively.	
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 VitroGnost cover glass.

Results

Nuclei - blue

Cytoplasm - shades of pink (pink-red when staining with Eosin Contrast).

Collagen, elastin, erythrocytes - yellow-orange (red-orange when staining with Eosin Contrast).

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 M in a tightly closed original package 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. Lillie, R.D. (1977): Conn's Biological Stains, 9th ed., Baltimore, Williams and Wilkens Co.
2. Mayer, P. (1891): Über das Färben mit Hämatoxzlin. Z. Wiss. Mikrosk. p8 337-341.
3. Mayer, P. (1904): Notiz über Hämatein und Hämalau, Y. Wiss. Mikrosk., p20 409-4011.

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	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile				

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