HEMATOXYLIN M

IVD In vitro diagnostic medical device CE

Modified hematoxylin acc. to Mayer for nuclear staining

Reagent used for progressive staining in histopathology and contrast staining in immunohistochemistry

INSTRUCTIONS FOR USE

REF Product code: HEMM-OT-30 (30 mL) HEMM-OT-100 (100mL) HEMM-OT-110 (10x100mL) HEMM-OT-500 (500mL) HEMM-OT-1L (1000mL) HEMM-OT-2.5L (2500mL)

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-eosin (H-E) staining and contrast staining 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 (*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. BioGnost's Mayer modified hematoxylin 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 contrast staining in immunohistochemistry. Contains optimally oxidized hematoxylin with sodium iodate, chloral hydrate stabilizer and antioxidants.

Other products and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration 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, for instance limonene-based BioNene or aliphatic hydrocarbon-based BioClear New agent
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax 52/54, BioWax 56/58, BioWax Blue, BioWax Micro, BioWax Plus 56/58
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of BioGnost's glass slides
- Bluing agent, such as BioGnost's Scott's solution or Bluing reagent
- Covering and mounting media such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua, Canada Balsam, or MountQuick Tube
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- Immersion oils such as BioGnost's Immersion oil, Cedarwood oil, Immersion oil types 37, A, B, FF and NVH
- Contrast staining reagents, such as BioGnost's eosin solutions: Eosin aqueous 0.5%, Eosin aqueous 1%, Eosin 0.5% alcoholic, Eosin Contrast

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 (BioNene, BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax 52/54, BioWax 56/58, BioWax Blue, BioWax Micro, BioWax Plus 56/58).
- Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

Hematoxylin-eosin (H-E) staining procedure, <u>progressive</u>

- Deparaffinize the section using xylene (BioClear) or a xylene substitute (BioNene or BioClear New), then rehydrate the section through series of descending alcohol solutions (Histanol 100, Histanol 95, Histanol 80 and Histanol 70) to distilled or demineralized water.
- Stain the section with Hematoxylin M solution by exposing it for 5 min or until an optimal level of staining is achieved.
  Note: In the case of subsidence in the solution or a formation of metallic glow on the surface, reagent should be filtrated before use
- Rinse the section under running water for 3-5 min.
- Stain the section with one of the contrasting solutions (Eosin Y 0.5% aqueous, Eosin Y 1% aqueous, Eosin Y 0.5% alcoholic, Eosin Contrast) by immersing it for 60 seconds to 2 min or until an optimal level of staining is achieved.
  Note: Staining the sections in Eosin Y 0.5% alcoholic and Eosin Contrast causes intensive eosinophil color to show much faster (in under 60 seconds time). Exposition time for Eosin Y 0.5% and Eosin 1% aqueous is 2 min and 90 seconds, respectively
- Immerse the section in water until it turns blue, that is until the excessive amount of eosinophil color is washed off.
- Dehydrate the section by immersing it into two exchanges of a 95% alcohol solution (Histanol 95). Repeat 10-15 times.
- Completely dehydrate the section by immersing it into three exchanges of a 100% alcohol solution (Histanol 100). Repeat 10-15 times.
- Clear the section by immersing in two exchanges of xylene (BioClear) or a xylene substitute (BioNene, BioClear New). Repeat 10-15 times.
- Mount with appropriate medium. BioMount, BioMount High, BioMount M, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, Canada Balsam, or MountQuick Tube if BioClear xylene was used. 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 - 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 use. 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 expired solutions should be taken care of as a 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

Hematoxylin M should be stored at temperature between 15 °C and 25 °C. Do not keep in cold places, do not freeze and avoid exposing to direct sunlight. Production date 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 Hämalaun, Y. Wiss. Mikrosk., p20 409-4011

HEMM-OT-X, V9-EN4, 21.10.2014., IŠP/VR

	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	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						