BIOGNOST®

HEMATOXYLIN ML

IVD *In vitro* diagnostic medical device CE

Modified hematoxylin acc. to Mayer-Lillie for nuclear staining
Strong intensity new generation reagent for progressive and regressive staining in histopathology

INSTRUCTIONS FOR USE

REF Catalogue number: HEMML-OT-30 (30 mL) HEMML-OT-100 (100 mL) HEMML-OT-500 (500 mL) HEMML-OT-1L (1000 mL) HEMML-OT-2.5L (2500 mL)

Introduction

BioGnost's Hematoxylin ML is one of the formulations of hematoxylin used in histopathology and cytology for a more precise nuclear cell staining. It is applied both progressively and regressively in a routine hematoxylin and eosin (HE) staining.

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. Unlike the hematoxylin acc. to Mayer, modified hematoxylin acc. to Mayer-Lillie contains 5 times greater concentration of hematoxylin, it is glycerol-stabilized, and a low pH value contributes to strong selectivity of color coding according to chromatin. By using the progressive method of staining, the microscopic samples are exposed to modified hematoxylin acc. to Mayer-Lillie long enough to stain the nucleus only, while regressive staining can also dye mucin (as well as the nucleus). BioGnost's Hematoxylin ML provides outstanding results in staining the nuclear membrane, nucleoplasm, nucleolus and mucin if the regressive method is used.

Product description

- **HEMATOXYLIN ML** - Reagent for both progressive and regressive nuclear staining in histopathology and cytology. Contains optimally oxidized hematoxylin with sodium iodate, glycerol 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 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, BioWax Micro.
- 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
- Differentiation agent, such as BioGnost's Acid alcohol
- Bluing agents, such as BioGnost's Scott's solution or Bluing reagent
- 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 C, BioMount Aqua
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- 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 μm 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 using Hematoxylin ML	3-5 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/demineralized water until dye is no longer being released from the preparation	
7.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: End 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 - 2 minutes
	Note: Staining the sections in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposition time for eosin aqueous solutions is 90 seconds to 2 minutes	
11.	Rinse under tap water Note: If an eosin alcoholic solution is used as a contrast dye, skip this step	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.

Hematoxylin and eosin (HE) staining procedure, <u>regressive</u>

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 using Hematoxylin ML	4-8 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.	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 section is treated with the differentiation agent for too long	
8.	Rinse in distilled 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 sections under tap water for 3-5 minutes	
10.	Immerse the sections in distilled/demineralized water	
11.	If alcoholic eosin solution is used, immerse the sections in 95% alcohol (Histanol 95). Skip this step if aqueous eosin solution is used	
12.	Stain with one of eosin contrast solutions until the section is optimally stained	15 seconds - 2 minutes
	Note: Staining the sections in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposition time for eosin aqueous solutions is 90 seconds to 2 minutes	
13.	Rinse under tap water Note: If an eosin alcoholic solution is used as a contrast dye, skip this step	2 min
14.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
15.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
16.	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, collagen, muscle fibers, mucin - shades of pink (red when staining with Eosin Contrast)
Erythrocytes - red

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 ML 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. Baker, J.R. (1962): Experiments on the action of mordants. 2. Aluminium-hematein. Q.J.Microsc. Sci. 103: 493-517.
2. Lillie, R.D. (1965): Histopathologic Technic and Practical Histochemistry, 3rd ed., New York, McGraw-Hill Book Co.
3. Lillie, R.D. et Fullmer H.M. (1976): Histopathologic Technic and Practical Histochemistry, 4th ed., New York, McGraw-Hill Book Co.
4. Lillie, R.D. et al. (1976): Nuclear stains with soluble metachrome mordant lake dyes. Histochemistry p49 23-35.
5. Mayer, P. (1904): Notiz über Hämatein und Hämalaun, Y. Wiss. Mikrosk., p20 409-4011.

HEMML-OT-X, V14-EN13, 26 April 2022, KB/IŠP

	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
	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						