BIOGNOST®

HEMATOXYLIN ML

CE IVD *In vitro* diagnostic medical device Classification according to Regulation (EU) 2017/746 - **Class A** product

Modified hematoxylin for nuclear staining acc. to Mayer-Lillie

Reagent for progressive and regressive staining in histopathology

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC30708STARVF				
EMDN code	W01030708				
REF **Catalog number**	**Volume**	**UDI-DI**	REF **Catalog number**	**Volume**	**UDI-DI**
HEMML-OT-100	100 mL	03858888821128	HEMML-OT-1L	1000 mL	03858888820022
HEMML-OT-500	500 mL	03858888821135	HEMML-OT-2.5L	2500 mL	03858888821142

Intended use and test principle

BioGnost's Hematoxylin ML is one of the hematoxylin formulations used in histopathology for precise staining of cell nuclei. Hematoxylin ML is applied using the progressive and regressive method in routine hematoxylin-eosin (HE) staining in histology. Hematoxylin is obtained by extraction from logwood (*Haematoxylon campechianum L.*). By oxidizing hematoxylin into hematein and binding it 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 a characteristic blue color. Unlike Mayer's hematoxylin, the Mayer-Lillie modification contains a 5x higher concentration of hematoxylin, is stabilized with glycerol, and the low pH value contributes to the strong selectivity of the stain for chromatin. With the progressive staining method, microscopic specimens are exposed to Mayer-Lillie hematoxylin long enough to stain only the nucleus, while with regressive staining, mucin can also be stained in addition to the nucleus. BioGnost's Hematoxylin ML produces excellent staining results for the nuclear membrane, nucleoplasm, nucleolus, and mucin when the regressive staining method is performed.

Product description

- **HEMATOXYLIN ML** – Reagent for progressive and regressive nuclear staining in histopathology. Contains optimally oxidized hematoxylin with sodium iodate, glycerol as a stabilizer, and antioxidants

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
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax 56/58, BioWax Plus 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 DPX New, 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
- Contrast staining reagents such as BioGnost's eosin solutions
- Differentiating reagent, such as BioGnost's Acid Alcohol
- Nuclei bluing reagents such as 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 micron thin slices 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.	Rehydration in distilled/demineralized water	2 min
5.	Stain with Hematoxylin ML	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 color 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	
10.	Stain with one of the eosin counterstain solutions until optimal staining of the preparation is achieved	15 s to 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 Note: If an alcoholic eosin solution is used as a counterstain, skip this step.	2 min
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 to the slide. 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.

***** The procedure for automatic staining with the hematoxylin-eosin (HE) method is available in the Instructions for Use of BioGnost products Hem Diff, Hem Diff Strong, and BioBluing buffer.

Manual hematoxylin-eosin (HE) staining procedure, regressive

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 minutes
4.	Rehydration in distilled/demineralized water	2 minutes
5.	Stain with Hematoxylin ML	4-8 minutes
	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 color from the slide stops	
7.	Differentiation with Acid Alcohol	3-10 dips
	Note: This step removes excess hematoxylin from the nucleus and cytoplasm. If the sample has been treated with a differentiating agent for too long, the nuclei may become discolored.	
8.	Rinse in distilled/demineralized water	
9.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 minute
	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	
10.	Immerse the slide in distilled/demineralized water	
11.	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	
12.	Stain with one of the eosin counterstain solutions until optimal staining of the preparation is achieved	15 seconds to 2 minutes
	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.	
13.	Rinse under running tap water Note: If an alcoholic eosin solution is used as a counterstain, skip this step.	
14.	Dehydration in 95% alcohol (Histanol 95)	2 exchanges of 10-15 dips
15.	Dehydration in 100% alcohol (Histanol 100)	3 exchanges of 10-15 dips
16.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium to the slide. 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, mucin - shades of pink (shades of red when staining with Eosin Contrast)
Erythrocytes - red

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in BioGnost's instructions for use may cause variations in the 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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, staining 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, instructions for use, 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 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: 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 49: pp. 23-35.
5. Mayer, P.(1904): Notiz über Hämatein und Hämalaun, Y. Wiss. Mikrosk., 20, pp. 409-4011.

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.
	P305+P351+P338	IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

HEMML-IFU_ENV14, 09.04.2026., IŠP

	Manufacturer	LOT	Batch code		Consult Instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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

Version	Description / reason for change	Date
14	Revised acc. to Regulation (EU) 2017/746 - IVDR	09.04.2026.