

# HEMATOXYLIN COL

IVD *In vitro* diagnostic medical device



## Modified Hematoxylin acc. to Cole for progressive and regressive nuclear staining in histopathology, contains iodine

### INSTRUCTIONS FOR USE

REF Catalogue number: HEMCO-OT-100 (100 mL) HEMCO-OT-500 (500 mL) HEMCO-OT-1L (1000 mL)

#### Introduction

BioGnost's Hematoxylin COL is a formulation of hematoxylin used in histopathology for nuclear cell staining. It can be used instead of Hematoxylin M (Mayer's Hematoxylin). Unlike Hematoxylin M (used only in progressive staining method) Hematoxylin COL is used in both progressive and regressive staining methods.

Hematoxylin is extracted from logwood (*Haematoxylon 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 COL is oxidized with iodine and ready for instant use.

#### Product description

- **HEMATOXYLIN COL** - Reagent for progressive and regressive nuclear staining in histopathology. Contains optimally oxidized hematoxylin

#### 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: Eosin Y 0.2 aqueous, Eosin Y 0.5 aqueous, Eosin Y 1% aqueous, Eosin Y 2% aqueous, Eosin Y 0.5% alcoholic, Eosin Contrast

#### Preparing histological sections for staining

- Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), 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 56/68, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6  $\mu$ m slices and place them on a VitroGnost glass slide.

#### Hematoxylin and eosin (HE) staining procedure, regressive

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 COL	10 min
	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.	Rinse shortly in distilled (demi) water	
7.	Differentiate using Acid alcohol	10 dips
	Note: This step removes excessive hematoxylin. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long.	
8.	Rinse in distilled (demi) water	
9.	Blue using Scott's solution or Bluing reagent	1 min
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	
10.	Rinse in distilled (demi) water	
11.	Stain with Eosin Y counterstaining solution	15 seconds - 2 minutes
	Note: Staining the sections in Eosin Y 0.5% alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds time). Exposition time for Eosin Y aqueous solutions is 2 min and 90 seconds, respectively.	
12.	Rinse in distilled (demi) water	
9.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
10.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
11.	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 a VitroGnost cover glass.

## 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.

## Results

Nuclei - blue

Cytoplasm, collagen, muscle fibers, erythrocytes - shades of pink (red when staining with Eosin Contrast)

## 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 COL 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. p103 493-517.
2. Conn, J. (1977): Biological Stains, 9<sup>th</sup> ed., Baltimore: Williams and Wilkens Co.
3. Bancroft, J.D. and Stevens A. (2008): Theory and practice of histological techniques, 6<sup>th</sup> ed., Churchill Livingstone, Edinburgh & London, UK

HEMCO-X, V5-EN4, 10 July 2019, AK/IŠP

	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				



BIOGNOST Ltd.  
Medjugorska 59  
10040 Zagreb  
CROATIA  
www.biognost.com

