EOSIN CONTRAST PLUS

IVD *In vitro* diagnostic medical device CE

Modified alcoholic solution for cytoplasmic counterstaining

Reagent contains Eosin Y, Phloxine B, and Biebrich Scarlet for additional effect of cytoplasmic counterstaining

INSTRUCTIONS FOR USE

REF Catalogue number: EOYKP-OT-1L (1000 mL) EOYKP-OT-2.5L (2500 mL)

Introduction:

BioGnost's Eosin Contrast Plus is a reagent which is commonly used as a contrast dye for hematoxylin in the histological staining method, the hematoxylin and eosin (HE) staining. This method achieves better cellular structure visualization and differentiation. The microscopic samples' nuclei are stained blue using hematoxylin, then they are stained various shades of pink and red using cytoplasm eosin. Unlike standard aqueous eosin solutions, alcoholic solution of Eosin Y, Phloxine B, and Biebrich Scarlet (modification of Meter's Eosin) stain sections more intensely pink and red. Eosin Y, Phloxine B, and Blebrich Scarlett are anion dyes that stain erythrocytes bright red, and it also stains basic cellular components, such as cytoplasm, collagen, and muscle fibers.

Product description:

- **EOSIN CONTRAST PLUS** – Alcoholic solution of Eosin Y, Phloxine B, and Biebrich Scarlet for more intensive cytoplasmic counterstaining. Contains stabilizers for longer shelf life.

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
- Nuclei staining reagents, such as BioGnost's hematoxylin solutions: Hematoxylin H, Hematoxylin ML, Hematoxylin G1, Hematoxylin G2, Hematoxylin G3 and Hematoxylin M

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 56/58, 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 M, Hematoxylin ML, Hematoxylin G1, G2, or Hematoxylin H	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 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 section in distilled/demineralized water.	
9.	Immerse the section in 95% alcohol (Histanol 95)	30 seconds
10.	Stain using Eosin Contrast PLUS solution	up to 15 seconds
11.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
12.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
13.	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.

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, Hematoxylin G3, or Hematoxylin H	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.	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 section in distilled/demineralized water.	
11.	Immerse the section in 95% alcohol (Histanol 95)	30 seconds
12.	Stain using Eosin Contrast PLUS solution	up to 15 seconds
13.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
14.	Dehydrate using 100% alcohol (Histanol 100)	3 exchanges, 10-15 dips
15.	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.

Result

Nuclei - blue
Cytoplasm, collagen, muscle fibers, erythrocytes - hues of pink

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 Eosin Contrast Plus in a tightly closed original package at temperature between +15°C and +25°C. Do not keep in cold 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. Bruce-Gregorios, J.H. (1974): Histopathologic Techniques, *IMC Press Inc.,* Quezon City, Philippines.
2. Cook, D.J. (2009): *Cellular Pathology: An introduction to techniques and applications.* 2nd ed., Scion Publishing Ltd., Bloxham.
3. Gurr, E. (1971): *Synthetic dyes in biology, medicine and chemistry*. Academic Press, London.

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	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						