BIOGNOST®

EOSIN Y 1% AQUEOUS

IVD In vitro diagnostic medical device CE

1% eosin yellowish water solution for cytoplasmic contrast staining

More intense contrast cytoplasmic staining reagent commonly used in classic hematoxylin-eosin staining (H-E)

INSTRUCTIONS FOR USE

REF Product code: EOY-10-OT-1L (1000 mL) EOY-10-OT-2.5L (2500 mL)

Introduction:

BioGnost's Eosin Y 1% is an aqueous reagent which is commonly used as a contrast dye for hematoxylin in the histological staining method, the hematoxylin-eosin (H-E) staining. This method achieves better cellular structure visualization and differentiation. The microscopic samples' nuclei are stained blue using hematoxylin, and then cytoplasms are stained in various shades of pink using eosin dye. Eosin Y is a fluorescein derivative. As color powder it can be used as a reagent mixture often used in histological, but also in cytological methods of staining, such as the Papanicolaou method in exfoliative cytology or for creating Romanowsky dyes. Eosin Y is anion dye which stains erythrocytes bright red, and it also stains basic cellular components, such as cytoplasm, collagen, and muscle fibers.

Product description:

- **EOSIN Y 1% AQUEOUS** - Contrast cytoplasmic staining reagent contains stabilizers and a low concentration of fungicide.

Other products and reagents that may be used in staining:

- Fixative such as BioGnost's neutral buffered formalin: 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, 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 types 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 and mounting media such as BioGnost's BioMount, BioMount Aqua, BioMount C, BioMount DPX, BioMount DPX Low, BioMount DPX High, BioMount M, BioMount New, Canada Balsam, MountQuick Tube
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- Histopathology staining reagents, such as BioGnost's hematoxylin solutions: Hematoxylin H, Hematoxylin ML, Hematoxylin G3 and Hematoxylin M
- Immersion oils such as BioGnost's Cedarwood oil, Immersion oil, Immersion oil types A, B, NVH, FF and 37

Preparing the histological sections for staining

- Fixate the tissue sample tightly (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 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>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 H	8-15 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.	Bluing using Scott's solution or Bluing reagent	1 min
	Note: End the process of bluing after the nuclei turn blue	
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). Exposition time for eosin aqueous solutions is 2 min and 90 seconds, respectively.	
11.	Rinse under tap water	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 a VitroGnost cover glass.

Result:

Nucleus - blue
Cytoplasm, collagen, muscle fibers, erythrocytes - shades of pink (red 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 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 taken care of as a special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger for 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 Y 1% in a tightly sealed 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. Production date 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.

EOY-10-OT-X, V8-EN6, 04 July 2016., 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						