

# EOSIN Y 2% ALCOHOLIC

IVD *In vitro* diagnostic medical device



## 2% eosin yellowish alcoholic solution for cytoplasm counterstaining Reagent used in the classic hematoxylin and eosin staining

### INSTRUCTIONS FOR USE

REF Product code: EOYA-20-OT-1L (1000 mL)

EOYA-20-OT-2.5L (2500 mL)

#### Introduction

BioGnost's Eosin Y 2% is an alcohol 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 using cytoplasm eosin. 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 2% ALCOHOLIC** - Alcoholic reagent for cytoplasm counterstaining

#### Other slides 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, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro
- Glass slides used in histology, pathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR, or one of 30 (and more) BioGnost's glass slides
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua, Canada Balsam or MountQuick Tube medium
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and B
- Histopathology 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 PLUS 56/58)
- 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, 10 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	4-6 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.	Rinse using distilled (demi) water until dye is no longer being released from the section.	
7.	Differentiate using differentiation agent (Acid alcohol).	
	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 the section with distilled (demi) water until the surface of the section becomes homogenized	
9.	Bluing using Scott's solution or Bluing reagent	
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	
10.	Rinse the section with distilled (demi) water	
11.	Stain the section using one of counterstain solutions (Eosin Y 0.5 aqueous, Eosin Y 1% aqueous, Eosin Y 0.5% alcoholic, Eosin Y 1% alcoholic, Eosin Y 2% alcoholic Eosin Contrast).	15-90 seconds
	Note: If the alcohol solution of eosin is used, the section should be treated with a 95% alcohol solution (Histanol 95) by immersing it in the solution for 30 seconds. 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.	Dehydrate using 95% alcohol (Histanol 95)	3 exchanges, 2 min each
13.	Completely rehydrate by using 100% alcohol (Histanol 100)	3 exchanges, 2 min each
14.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 5 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 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 Y 2% alcoholic 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*. 2<sup>nd</sup> ed., Scion Publishing Ltd., Bloxham.
3. Gurr, E. (1971): *Synthetic dyes in biology, medicine and chemistry*. Academic Press, London.

EOYA-20-X, V1-EN1, 17 September 2015, IŠP/VR

	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				



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