

# EOSIN Y INSTANT, AQUEOUS

# EOSIN Y INSTANT, ALCOHOLIC

IVD *In vitro* diagnostic medical device CE

## For preparing 1 liter of instant solution of yellowish eosin for cytoplasmic counterstaining

### INSTRUCTIONS FOR USE

REF Catalogue number: EOYI-AQ-1L (1000 mL) EOYI-ALC-1L (1000 mL)

#### Introduction

Eosin Y solutions are regularly used as counterstains to hematoxylin in histology staining method, the HE (hematoxylin-eosin) 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. BioGnost's Eosin Y Instant, aqueous and alcoholic preparations are very easily dissolved in water or in 70% alcohol. They are ready for instant use.

#### Product description

- **EOSIN Y INSTANT, AQUEOUS** – mixture for preparing Eosin Y Instant in aqueous medium.
- **EOSIN Y INSTANT, ALCOHOLIC** – mixture for preparing Eosin Y Instant in alcoholic medium.

#### Preparing Eosin Y Instant

##### a) aqueous solution

1.	Fill the adequate container with 1 L of distilled (demi) water
2.	Carefully add contents of 1 bottle of Eosin Y Instant, aqueous.
3.	Mix for 1-2 minutes until the contents are completely dissolved.
4.	Use immediately or keep in a tightly closed container.
5.	Keep Eosin Y Instant, aqueous in a tightly closed original package at room temperature. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Shelf life of prepared Eosin Y Instant is 4-6 months.

##### b) alcoholic solutions

1.	Fill the adequate container with 1 L of 70% ethyl alcohol. For preparation of 70% ethyl alcohol, mix 700 ml of absolute ethyl alcohol with 300 ml of distilled (demi) water. DO NOT USE ONLY WATER.
2.	Carefully add contents of 1 bottle of Eosin Y Instant, alcoholic.
3.	Mix for 15-60 minutes until the contents are completely dissolved. Due to flammability of the solvent, do not heat the solution to achieve faster dissolution!
4.	Use immediately or keep in a tightly closed container.
5.	Keep Eosin Y Instant, alcoholic in a tightly closed original package at room temperature. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Shelf life of prepared Eosin Y Instant, alcoholic is 4-6 months.

#### 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 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 DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- Nuclei staining reagents, such as BioGnost's hematoxylin solutions: Hematoxylin Instant, 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 Instant, 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.	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 sections in distilled/demineralized water.	
9.	Stain using Eosin Y Instant aqueous or alcoholic solution	15 seconds to 2 min
	Note: Staining the sections in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposition time for eosin aqueous solutions is 90 seconds to 2 minutes.	
10.	Rinse under tap water	2 min
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, 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 Instant, 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 sections in distilled/demineralized water.	
11.	Stain using Eosin Y Instant aqueous or alcoholic solution	15 seconds to 2 min
	Note: Staining the sections in eosin alcoholic solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposition time for eosin aqueous solutions is 90 seconds to 2 minutes.	
12.	Rinse under tap water	2 min
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.

#### Results

Nuclei - blue

Cytoplasm, collagen, muscle fibers, erythrocytes - hues of pink when staining with Eosin Y Instant, aqueous  
- hues of pink to red when staining with Eosin Y Instant, alcoholic

#### 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 Instant, aqueous and 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.

EOYI-X, V4-EN4, 12 May 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 in vitro diagnostic use only		Keep in dry place		Caution - fragile				

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