BIOGNOST®

EOSIN Y 0.5% ALCOHOLIC

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

0.5% Eosin yellowish alcoholic solution for counterstaining cytoplasm

Reagent used in classical hematoxylin and eosin (HE) staining

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI**
EOYA-05-OT-1L	1000 mL	03858888820107
EOYA-05-OT-2.5L	2500 mL	03858890001587

Intended use and test principle

BioGnost's Eosin Y 0.5% is an alcoholic reagent regularly used as a contrast dye to hematoxylin in the histological staining method, hematoxin and eosin (HE) staining. With this method, in order to achieve better visualization and differentiation of cellular structures, the nuclei of microscopic samples are first stained blue with hematoxylin, and then the cytoplasm is stained several shades of pink with eosin. Eosin Y is a derivative of fluorescein and as a powder dye it can be used for the preparation of reagents often used in histological and cytological staining methods such as the Papanicolaou method in exfoliative cytology or for obtaining Romanowsky stains. Eosin Y is an anionic dye that, in addition to the basic components of cells, such as cytoplasm, collagen and muscle fibers, also stains erythrocytes in a bright red color. Unlike aqueous solutions of Eosin Y dye, alcoholic solutions dye the cell component with a more intense dye.

Product description

- **Eosin Y 0.5% ALCOHOLIC** – Alcoholic reagent for cytoplasmic counterstaining, contains stabilizers

Additional reagents and materials that can be used in staining

- Fixatives, such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX New, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- Reagents for staining nuclei such as BioGnost Hematoxylin solutions (H, ML, G1, G2, G3 and Hematoxylin M)
- Differentiating reagent, such as BioGnost's Acid Alcohol.
- Nuclei bluing reagents such as BioGnost's Bluing reagent or Scott's solution

Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 micron thin slices and mount on a VitroGnost microscope slide

Note

Make sure that the part of the slide with the sample is completely immersed in the appropriate solution or reagent in each step.

Hematoxylin-eosin (HE) manual* staining procedure, progressive

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Staining with Hematoxylin M, Hematoxylin ML, Hematoxylin G1, G2 or Hematoxylin H	3-5 min
	Note: If there has been precipitation in the solution or the formation of a metallic sheen on the surface, the reagent must be filtered before use	
6.	Immerse the slide in distilled/demineralized water until the release of color from the slide stops	
7.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: Stop bluing after the nuclei turn blue. In the absence of Scott's solution or Bluing reagent, rinse the slides under running water for 3-5 minutes	
8.	Immerse the slide in distilled/demineralized water	
9.	Immerse the slide in 95% alcohol (Histanol 95)	30 sec
10.	Staining with Eosin 0.5% alcoholic solution	15 sec to 1 min
11.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges of 10-15 dips
12.	Dehydrate in 100% alcohol (Histanol 100)	3 exchanges of 10-15 dips
13.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

***** The procedure for automatic staining with the hematoxylin-eosin (HE) method is available in the Instructions for Use of BioGnost products Hem Diff, Hem Diff Strong and BioBluing buffer.

Hematoxylin-eosin (HE) manual* staining procedure, regressive

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min

3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Staining with Hematoxylin ML, Hematoxylin G3 or Hematoxylin H	4-8 min
	Note: If there has been precipitation in the solution or the formation of a metallic sheen on the surface, the reagent must be filtered before use	
6.	Immerse the slide in distilled/demineralized water until the release of color from the slide stops	
7.	Differentiation with Acid alcohol	3-10 dips
	Note: This step removes excess hematoxylin from the nucleus and cytoplasm. If the sample has been treated with a differentiating agent for too long, the nuclei may become discolored	
8.	Rinse in distilled water	
9.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: Stop bluing after the nuclei turn blue. In the absence of Scott's solution or Bluing reagent, rinse the slides under running water for 3-5 minutes	
10.	Immerse the slide in distilled/demineralized water	
11.	Immerse the slide in 95% alcohol (Histanol 95)	30 sec
12.	Staining with Eosin 0.5% alcoholic solution	15 sec to 1 min
13.	Dehydrate in 95% alcohol (Histanol 95)	2 exchanges of 10-15 dips
14.	Dehydrate in 100% alcohol (Histanol 100)	3 exchanges of 10-15 dips
15.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

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Result

Nuclei - blue color

Cytoplasm, collagen, muscle fibers, erythrocytes - shades of pink

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. It is necessary to follow the manufacturer's instructions for use. To avoid errors, staining and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards.

If a serious incident occurs during use or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use and in the safety data sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The production date and expiration date are printed on the product label.

References

1. *Bruce-Gregorios, JH (1974): Histopathologic Techniques, IMC Press Inc., Quezon City, Philippines.*.
2. Cook, DJ (2009): *Cellular Pathology: An introduction to techniques and applications.* 2n.d ed., Scion Publishing Ltd., Bloxham.
3. Gurr, E. (1971): *Synthetic dyes in biology, medicine and chemistry*. Academic Press, London.

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapour.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

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	Manufacturer	LOT	Batch code		Consult Instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
9	Revised acc. to Regulation (EU) 2017/746 - IVDR	08.04.2026