EOSIN Y 0,5% ALCOHOLIC

IVD In vitro diagnostic medical device CE

0,5% eosin yellowish alcohol solution for cytoplasmic contrast staining

Reagent used in the classic hematoxylin-eosin staining

INSTRUCTIONS FOR USE

REF Product code: 01EOYA051000 (1000mL) 01EOYA052500 (2500mL)

Introduction

Eosin Y 0,5% is an alcohol 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 citoplasms 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 0,5% ALCOHOLIC** - 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 neutral buffered formalin: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as xylene or a substitute, for instance limonene-based or aliphatic hydrocarbon-based agent
- Infiltration and fitting agent, such as granulated paraffin Lab-O-Wax 52/54, Lab-O-Wax 56/58, Lab-O-Wax Plus 56/58
- High-quality glass slides for use in histopathology and cytology, such as or one of more than 30 types of glass slides
- Differentiation agent, such as Acid alcohol
- Bluing agents, such as Scott's solution or Bluing reagent
- Covering and mounting media such as HistoMount, HistoMount High, HistoMount M, HistoMount New, HistoMount DPX, HistoMount DPX High, HistoMount DPX Low, HistoMount C, HistoMount Aqua, Canada Balsam, or MountQuick Tube
- cover glass, dimensions range from 18x18 mm to 24x60 mm
- Histopathology staining reagents, such as hematoxylin solutions: Hematoxylin H, Hematoxylin ML, Hematoxylin G3 and Hematoxylin M
- Immersion oils such as Immersion oil, Cedarwood oil, Immersion oil types 37, A, B, FF and NVH

Preparing the histological sections for staining

- Fixate the tissue sample tightly (Formaldehyde NB 4%, Formaldehyde NB 10% or Formaldehyde NB 37%), 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 or in a xylene substitute.
- Infiltrate and fit the sample in paraffin (Lab-O-Wax 52/54, Lab-O-Wax 56/58, Lab-O-Wax Plus 56/58).
- Cut the paraffin block to 4-6 µm slices and place them on a glass slide.

Hematoxylin-eosin (H-E) staining procedure, regressive

- Deparaffinize and dehydrate the section through series of ascending alcohol solutions (Histanol 95, Histanol 80, and Histanol 70).
- Rinse the section with distilled or demineralized water until the surface of the preparation becomes homogenized.
- Stain the section with a Hematoxylin H solution by immersing it in the solution for 4-5 min.
  Note: In the case of sediment in the solution or a formation of metallic glow on the surface, reagent should be filtrated before use.
- Rinse the section with distilled or demineralized water until dye is no longer being released from the preparation.
- Remove excessive dye by using a 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.
- Rinse the section with distilled or demineralized water until the surface of the section becomes homogenized.
- Previously stained section with red nuclei should be treated with a bluing agent (Scott's solution, Bluing reagent).
  Note: End the process of bluing after the nuclei turn blue.
- Wash the section with distilled or demineralized water.
- Stain the section with one of the contrasting solutions (Eosin Y 0.5% aqueous, Eosin Y 1% aqueous, Eosin Y 0.5% alcoholic, Eosin Contrast) by immersing it in the solution for 15 seconds to 90 seconds.
  Note: In case of using the eosin alcohol solution, the preparation should first be treated with a 95% alcohol solution (Histanol 95) by immersing it in the solution for 30 seconds, and time of exposure to Eosin Y 0.5% alcoholic should be extended to 1-2 min.
- Dehydrate the section with three exchanges of a 95% alcohol solution (Histanol 95) for 2 min.
- Completely dehydrate the section with three exchanges of a 100% alcohol solution (Histanol 100) for 2 min.
- Mount with appropriate medium. HistoMount, HistoMount High, HistoMount M, HistoMount DPX, HistoMount DPX High, HistoMount DPX Low, HistoMount C, Canada Balsam, or MountQuick Tube if xylene was used. If xylene substitute was used, the appropriate covering agent is HistoMount New.
- Cover the section with a 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 material safety data sheet.

Storing, stability and expiry date

Keep Eosin Y 0,5% alcoholic 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.

EOYA-05-OT-X, V6-EN4, 2 Feb 2015, IŠP/VR

	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	European Conformity
	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				

Histo-Line Laboratories srl
V. G. Di Vittorio 30
20090 Pantigliate (MI)
ITALY
www.histoline.com

CE