

# HISTANOL EP 9010

IVD *In vitro* diagnostic medical device



## Rehydrating/dehydrating agent

### Mixture of denatured ethanol and isopropanol for use in histology and cytology

#### INSTRUCTIONS FOR USE

REF Catalogue number: HEP91-1L (1000 mL)    HEP91-5L(5000 mL)    HEP91-10L (10000 mL)

#### Introduction

Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. Quality sample processing should be carried out in order to achieve good tissue and cellular structures visualization. Histological sample processing consists of a few steps, three of them consist of dehydration and rehydration. The first step consists of preparing the samples for infiltration and fitting in paraffin and cutting the paraffin blocks in thin slices. The second step consists of preparing the samples for staining. The final step consists of preparing the samples for mounting on the glass slide. Most of the fitting and infiltrating media (such as commonly used paraffin) will not permeate the water containing sample. Dehydration must be carried out first in order to achieve that. After adding the intermedium (a medium that enables permeating the sample using paraffin), fitting in paraffin, cutting it in thin slices and mounting them on a glass slide, the section will not deteriorate for a certain amount of time. However, paraffin should be removed from the section and it should be rehydrated before staining. Only then can the section be stained with histological dyes. A similar procedure is applied on cytological samples.

Most of dehydrating agents are alcohols. One of the agents is a mixture of denatured ethyl and isopropyl alcohol, i.e. BioGnost's Histanol EP 9010. Histanol EP 9010 is a transparent, colorless, and flammable liquid characteristic of its fast acting and high efficiency.

#### Product description

- **HISTANOL EP 9010** – Mixtures of denatured ethyl alcohol and isopropyl alcohol for dehydration/rehydration of tissue and cytology samples.

#### 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 and Histanol 95
- 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 52/54, 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
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- Reagent for nuclei staining such as BioGnost's Hematoxylin H
- Counterstaining reagents, such as BioGnost's eosin solutions

#### Preparing histological sections for staining

- Fix the tissue sample well (4% NB Formaldehyde, 10% NB Formaldehyde), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95, Histanol 96 and Histanol 100)
- Clear the sample with an intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New)
- Infiltrate and embed the sample in paraffin (BioWax 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue)
- Cut the paraffin block into 4-6  $\mu$ m slices and place them on a VitroGnost glass slide

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 EP 9010)	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	3-5 minutes
6.	Immerse the section in distilled or demineralized water until dye is no longer released from the section	
7.	Convert the purple/red stained nuclei into blue using Scott's solution or Bluing reagent	1 min
	Note: Stop the bluing process after the section visibly turns blue If no Scott's solution or Bluing reagent is available, rinse the sections under tap water for 3-5 minutes.	
8.	Stain with one of contrast eosin solutions until the section is optimally stained	15 seconds - 2 minutes
	Note: Staining the sections in alcoholic eosin solutions causes intensive eosinophil color to show much faster (in under 15 seconds' time). Recommended exposition time for aqueous eosin solutions is 90 seconds to 2 minutes.	
9.	Rinse under tap water	2 min
10.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 10-15 dips
11.	Dehydrate using 100% alcohol (Histanol EP 9010)	3 exchanges, 10-15 dips
12.	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 covering/mounting medium on the section. If BioClear xylene was used, use one of BioGnost's xylene-based mounting 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 - dark blue

Cytoplasm, collagen, elastin, erythrocytes - various shades of pink (when staining with Eosin Contrast the shade is red-pink)

### 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 Histanol EP 9010 in a tightly closed original package at temperature between +15°C and +25°C. Keep in dry 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. Carson, F.L. (1926): *Histotechnology: a self-instructional text*. 2<sup>nd</sup> ed., Singapore: American Society for Clinical Pathology.
2. Sheehan, D.C. et Hrapchak, B.B. (1980): *Theory and Practice of Histotechnology*, 2nd ed., St. Louise: CV Mosby Co.
3. Papanicolaou GN: Some improved methods for staining vaginal smears. *J Lab Clin Med*. 1941;26:1200-1205.
4. Papanicolaou GN: A new procedure for staining vaginal smears. *Science*. 1942;95:438-439.

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	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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