BIOGNOST®

HISTANOL 50/70/80/95/96/100

IVD *In vitro* diagnostic medical device CE

Rehydrating/dehydrating agent
50%, 70%, 80%, 95%, 96%, 100% denatured alcohol for use in histology

INSTRUCTIONS FOR USE

REF Catalogue number:	H50-1L (1000 mL)	H70-1L (1000 ml)	H80-1L (1000 ml)	H95-1L (1000 ml)	H96-1L (1000 ml)	H100-1L (1000 ml)
	H50-5L (5000 ml)	H70-5L (5000 ml)	H80-5L (5000 ml)	H95-5L (5000 ml)	H96-5L (5000 ml)	H100-5L(5000 mL)
	H50-10L (10000 mL)	H70-10L (10000 ml)	H80-10L (10000 ml)	H95-10L (10000 ml)	H96-10L (10000 ml)	H100-10L (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 them (and the most commonly used one) is denatured ethanol, which is the main component of BioGnost's Histanol. Histanol is a transparent, colorless, and flammable liquid characteristic of its fast acting and high efficiency.

Product description

- **HISTANOL 50, HISTANOL 70, HISTANOL 80, HISTANOL 95, HISTANOL 96, HISTANOL 100** - Denatured alcohol solutions used for dehydration/rehydration of tissue and cytological 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, 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
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- Reagent for nuclear staining, such as Hematoxylin H
- Counterstaining reagents, such as BioGnost's eosin solutions

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 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.	Make nuclei turn 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.	Stain with one of eosin contrast solutions until the section is optimally stained	15 seconds - 2 minutes
	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.	
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 100)	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 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 - 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 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-instrucional text*. 2nd 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.

H50-X–H100-X, V14-EN2, 01 July 2019, AK/IŠP

	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	European Conformity		BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com
	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					CE	