todylaboratories®

Microbiology Stains

CE IVD REF C15MS1012

Histanol 100
Instructions For Use

Rehydrating/dehydrating agent; 70%, 95%, 96%, 100% alcohol for use in histology
In vitro diagnostic medical device

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 sectioning 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, sectioning 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 ethanol, which is the main component of Histanol. Histanol is a transparent, colorless, and flammable liquid characteristic of its fast acting and high efficiency.

PRODUCT DESCRIPTION

HISTANOL 100 - Alcohol solutions used for dehydration/rehydration of tissue and cytological samples.

OTHER PREPARATIONS AND REAGENTS THAT CAN BE USED IN THIS METHOD, BUT ARE NOT A PART OF THE SET

- Fixatives such as neutral buffered formaldehyde solutions: Formaldehyde NB 4% or Formaldehyde NB 10%
- Clearing agents, such as BioClear xylene or a substitute, for instance BioNene on the limonene basis or BioClear New agent on the aliphatic hydrocarbons basis.
- Infiltration and fitting agent, such as granulated paraffin BioWax Plus, BioWax 56/68, BioWax Blue, BioWax Micro.
- Glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR, or adhesive glass slides, such as VitroGnost PLUS ULTRA, VitroGnost SIL or VitroGnost PLL.
- Differentiation agent, such as Acid alcohol
- Bluing agents, such as Scott's solution or Bluing reagent.
- Covering agents for microscopic sections and mounting cover glass, such as BioMount DPX, BioMount DPX Low, BioMount DPX High, BioMount M, BioMount New
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- Staining reagents used in histology and cytology, such as hematoxylin solutions: Hematoxylin H, Hematoxylin M, Hematoxylin ML, Hematoxylin HP, Hematoxylin G1, Hematoxylin G2, Hematoxylin G3
- Contrast staining reagents, such as eosin solutions: Eosin aqueous 0.5%, Eosin aqueous 1%, Eosin 0.5% alcoholic, Eosin Contrast

PREPARING THE HISTOLOGICAL SECTIONS FOR STAINING

- Fixate the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 95 and Histanol 100).
- Clear the sample with an intermedium; in xylene (BioClear) or in a xylene substitute (BioNene, BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6µm slices and place them on a VitroGnost glass slide.

HEMATOXYLIN-EOSIN (HE) STAINING PROCEDURE

- Deparaffinize the section using xylene (BioClear) or a xylene substitute (BioNene or BioClear New),then rehydrate the preparation through series of descending alcohol solutions (Histanol 100, Histanol 95, Histanol 80, Histanol 70 and Histanol 50).
- Rinse the section with distilled or demineralized water until the surface of the preparation becomes homogenized.
- Stain the section using one of the nuclear staining solutions (HematoxylinH, HematoxylinM, HematoxylinML, HematoxylinHP, HematoxylinG1, Hematoxylin G2, Hematoxylin G3) by immersing it in the solution for 4 to 5 min, or until an optimal staining is achieved.
- Rinse the section with distilled or demineralized water until dye is no longer being released from the preparation.
- Remove the excessive dye by using the differentiating agent (Acid alcohol) if necessary (regressive method).
- Rinse the section with distilled or demineralized water until the surface of the preparation becomes homogenized.
- Previously stained section with red nuclei should be treated with a bluing agent (Scott's solution, Bluing reagent).
- 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).

Note: If the alcohol solution of eosin is used, the preparation should be treated with a 95% or a 96% alcohol solution (Histanol 95, Histanol 96) by immersing it for 30 seconds.

- Dehydrate the section with three exchanges of a 95% or a 96% alcohol solution(Histanol 95, Histanol 96) for 2min.
- Completely dehydrate the section with three exchanges of a 100% alcohol solution (Histanol 100) for 2 min.
- Clear the section in xylene (BioClear) or in a xylene substitute (BioNene, BioClear New).
- Mount with appropriate medium (BioMount DPX, or BioMount M if BioClear xylene or a BioNene xylene substitute was used). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New).
- Cover the section with a Vitro Gnost 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 taken care of as a 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 the material safety data sheet.

STORING, STABILITY AND EXPIRY DATE

Keep Histanol in a tightly closed original package at a room temperature. 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. 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.

SYMBOL INDEX

	Manufacturer
IVD	For in vitro diagnostic use only
LOT	Lot number
	Valid until
	Storage temperature range
	Keep away from heat and sunlight
CE	European Conformity
REF	Product code
	Refer to supplied instructions
Σ	Number of tests in package
	Refer to the supplied documentation
	Caution - fragile
	Keep in dry place

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