HEMATOXYLIN P.T.A.

IVD In vitro diagnostic medical device CE

Synonims: Hematoxylin Phospotungstic Acid, Mallory P.T.A.H. reagent

INSTRUCTIONS FOR USE

REF Catalogue number: HPTA-OT-500 (500 mL) HPTA-OT-1L (1000 mL)

Introduction

Hematoxylin P.T.A. reagent is used for staining collagen, muscle tissue (excellent diferentiation between smooth and striated muscle tissue), Nemaline rods (present in certain skeletal muscle diseases), fibrin (visible in tissues with fresh damage, in acute inflammatory reactions), glial fibres (display of gliosis in central nervous system), certain elastic fibers, cartilage and bone matrix. Tungsten from the excessive phosphotungstic acid in the reagent binds all the available hematein and creates blue pigment that selectively stains skeletal striated muscles, fibrin, nuclei and certain other elements. The remainder of the phosphotungstic acid stains red-brown structures such as collagen. Hematoxylin P.T.A. is a component of Hematoxylin P.T.A. kit.

Product description

HEMATOXYLIN P.T.A. - reagent that contains hematoxylin and phosphotungstic acid for selective staining of tissue structures.

Example of use of Hematoxylin P.T.A. as a component of Hematoxylin P.T.A. kit

Other sections 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 52/54, BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro
- 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 C, BioMount Aqua
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- Other components of Hematoxylin P.T.A. kit: Potassium permanganate, 0.5% solution (cat. number: KP05-OT-100), Sulfuric acid, 0.5% solution (cat. number: SK05-OT-100), Oxalic acid, 1% solution (cat. number: OKS1-OT-100)

Preparing the histological sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), 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.

NOTE:
Apply the reagent so it completely covers the section.
In order to avoid the section to get dry, we recommend using incubation chamber/plate.

Procedure of staining histology samples by using Hematoxylin P.T.A. kit with four 100 mL reagents (HPTA-K-100)

Pour the reagents into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

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.	Mix equal volumes of Potassium permanganate, 0.5% solution and Sulfuric acid, 0.5% solution, mix briefly. Dip the section into the prepared solution.	5 min
	Note: dispose of the solution after use	
6.	Rinse in distilled (demi) water	
7.	Immerse into Oxalic acid, 1% solution	5 min
8.	Rinse in distilled (demi) water	
9.	Dip the section into Hematoxylin P.T.A. Note: if you wish to accelerate the staining process, heat Hematoxylin P.T.A. for 20 seconds in microwave oven (500 W), remove the solution from the oven, immerse the section and incubate for 15 minutes (outside the oven!). Macroscopically check for section coloration; in case of inadequate	incubate overnight at room temperature

	coloration, repeat the procedure until adequate coloration is achieved	
10.	Rinse in distilled (demi) water	3-4 seconds
11.	Differentiate in 95% alcohol	several rapid dips
12.	Dehydrate using 100% alcohol (Histanol 100)	2 min
13.	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.

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.

Results

Nuclei, fibrin, myofibril, astrocytoma, certain elastic fibers, myelin fibers, glial cells - dark blue
Collagen, cartilage, bone matrix - hues of brick red

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 Hematoxylin P.T.A. in a tightly closed original package at temperature between +15°C and +25°C. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

Culling, C.F.A. (1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
Lillie, R.D. (1945): Studies on selective staining of collagen with acid aniline dyes, J. Technical Methods, 25:1
Peers, J.H. (1941): A modification of Mallory's Phosphotungstic acid hematoxylin stain for formaldehyde-fixed tissue. *Arch. Pathol.* 32:446
Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

HPTA-OT-X, V3-EN3, 10 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						