BIOGNOST®

SULFURIC ACID, 0.5% SOLUTION

IVD *In vitro* diagnostic medical device CE

For use in special kits
INSTRUCTIONS FOR USE

REF Product code: SK05-OT-100 (100 mL)

Introduction

Sulfuric acid, 0.5% solution is a component of many special staining kits, such as Paraldehyde Fuchsin kit, Hematoxylin P.T.A. kit, Masson Fontana kit and Reticulin Contrast kit. Paraldehyde Fuchsin kit is used for visualization and detecting pathologic changes in elastic fibers, such as elastic tissue atrophy, loss or thinning of elastic tissue caused by atherosclerotic changes or vascular diseases. It is also used for staining pancreatic beta cells, mastocyte granules, mucin, cartilage, argentaffin granules and sperm acrosomes. Hematoxylin P.T.A. kit is used for better visualization of central nervous system, fibrin, but also primarily for differentiating between smooth and skeletal muscle tissue. Masson Fontana kit is used in a specific method for proving melanin and argentaffin granules in histology sections, based on reduction of silver nitrate to elemental silver. Reticulin Contrast kit is used for identification and easier visualization of argentaffin reticular fibers in connective tissue.

Product description

SULFURIC ACID, 0.5% SOLUTION - Sulfuric acid aqueous solution

Example of using Sulfuric acid, 0.5% solution in 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 Plus, BioWax 52/54, BioWax 56/68, BioWax Blue.
- Covering agent 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
- 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 (KP05-OT-100), Oxalic acid, 1% solution (OKS1-OT-100), Hematoxylin P.T.A. (HPTA-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 Plus, BioWax 52/54, BioWax 56/58, BioWax Blue).
- 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.

Histology sections staining procedure

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.	Apply 5 drops of Potassium permanganate, 0.5% solution and 5 drops of Sulfuric acid, 0.5% solution.	5 min
6.	Rinse in distilled (demi) water	
7.	Treat with 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. Macroscopically check for section coloration; in case of inadequate coloration, repeat the procedure until adequate coloration is achieved	incubate overnight at room temperature
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 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.

Result

Dark blue - nuclei, fibrin, myofibril, astrocytoma, certain elastic fibers, myelin fibers, glial cells
Hues of brick red - collagen, cartilage, bone matrix

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 Sulfuric acid, 0.5% solution in a tightly sealed original packaging at temperature of 15 to 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

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

SK05-OT-100, V1-EN1, 16 January 2023, KB/IŠP

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