4% FORMALDEHYDE, ALCOHOLIC SOLUTION

IVD *In vitro* diagnostic medical device CE

For staining with Reticulin and Reticulin Contrast kits
INSTRUCTIONS FOR USE

Introduction

4% Formaldehyde, alcoholic solution is a component of special staining kits such as Reticulin and Reticulin Contrast kits. Reticulin Contrast kit is used for detecting argentaffin reticulin fibers in connective tissues. Reticulin has supporting function in the body, it is found in the liver, spleen and kidneys. Reticulin fibers are clearly defined with healthy liver; necrotic and cirrhotic liver has discontinuous fibers. The test is based on silver depositions on reticulin fibers. The tissue sample must be oxidized with potassium permanganate. Silver is formed from ammonia solution containing silver nitrate and is deposited in the form of brown sediment on reticulin fibers. Formalin acts as reducing agent and accelerates the procedure. Unbound silver is washed away and removed by using sodium thiosulfate. Reticulin Contrast kit also contains gold chloride solution that stabilizes and tones the section's image. Reticulin Contrast kit contains Nuclear Fast Red (Kernechtrot) counterstain.

Product description

- **4% FORMALDEHYDE, ALCOHOLIC SOLUTION** – paraformaldehyde alcoholic solution.

Example of 4% Formaldehyde, alcoholic solution use as a Reticulin Contrast kit component

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 56/68, BioWax Blue, BioWax Micro.
- 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
- Other components of Reticulin Contrast kit: Potassium permanganate, 0.5% solution (KP05-OT-100), Sulfuric acid, 3% solution (SK3-OT-100), Oxalic acid, 1% solution (OKS1-OT-100), Ammonium iron sulfate (ASF-OT-100), Silver ammonium solution (SA-OT-100), Sodium thiosulfate, 5% solution (NT5-OT-100), Gold chloride, 0.2% solution (ZK02-OT -100), Nuclear fast red (Kernechtrot) reagent (KR-OT-100).

NOTE

Adhere to the following rules in order to achieve the best results:

- use distilled or demineralized high purity water **WITHOUT** any chlorine
- use completely clean laboratory glassware
- avoid contact between metal objects and solution (scissors, tweezers and so on)
- apply the reagent so it completely covers the section.
- if precipitate shows in *Ammonia iron sulfate, solution,* it should be filtered (the precipitate does not affect the staining quality)

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 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to **4-6** μm slices and place them on a VitroGnost glass slide.

Procedure for staining histology samples by using nine 100 mL reagent Reticulin Contrast kit (RET-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.	Prepare working solution: mix equal volumes of potassium permanganate and sulfuric acid solution. Note: Always prepare fresh working solution.	
6.	Immerse the section into working solution and let it react	5 min
7.	Rinse in distilled (demi) water	until the excessive reagent is washed off of the section
8.	Immerse into Oxalic acid, 1% solution	1 min
9.	Rinse thoroughly in distilled (demi) water twice	until the excessive reagent is washed off of the section
10.	Immerse into Ammonia iron sulfate, solution	3 min
11.	Rinse thoroughly in distilled (demi) water twice	until the excessive reagent is washed off of the section
12.	Immerse into Silver ammonia solution	3 min
13.	Rinse in distilled (demi) water	until the excessive reagent is washed off of the section
14.	Immerse into 4% formaldehyde, alcoholic solution	5 min

15.	Rinse thoroughly in distilled (demi) water twice	until the excessive reagent is washed off of the section
16.	Immerse into Gold chloride, 0.2% solution	2 min
17.	Rinse in distilled (demi) water	until the excessive reagent is washed off of the section
18.	Immerse into Sodium thiosulfate, 5% solution	2 min
19.	Rinse in distilled water	until the excessive reagent is washed off of the section
20.	Immerse into Nuclear Fast Red (Kernechtrot) reagent	5 min
21.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
22.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
23.	Dehydrate using 100% alcohol (Histanol 100)	2 min
24.	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.

Result

Reticular and nerve fibers - dark purple to black
Nuclei - pink to red
Collagen - ocher to brown-black
Background - light 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

4% Formaldehyde, alcoholic solution should be stored 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. Gomori, G. (1939): The effect of certain factors on result of silver impregnation for Reticulum fibers, Am. J. Path. , 15; 493-495
2. Gordon et Sweet, H. (1936): A rapid method for silver impregnation of reticulum, Am. J. Path., 12: 545-551

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	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						

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