

RETICULIN KIT

IVD *In vitro* diagnostic medical device



Seven-reagent kit for determining reticulin fibers

INSTRUCTIONS FOR USE

REF Catalogue number: RE-100T (for 100 tests)

RE-K-50 (7x50 mL)

RE-K-100 (7x100 mL)

Introduction

Reticulin kit is used for identification and easier visualization of argentaffin reticular fibers in connective tissue. 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.

Product description

- **RETICULIN KIT** - Seven-reagent kit for detecting reticular fibers

The kit contains:	100 tests (RE-100T)	7 x 50 mL (RE-K-50)	7 x 100 mL (RE-K-100)	Storage temperature
Potassium permanganate, 0.5% solution	30 mL (KP05-OT-30)	50 mL (KP05-OT-50)	100 mL (KP05-OT-100)	15-25°C
Sulfuric acid, 3% solution	30 mL (SK3-OT-30)	50 mL (SK3-OT-50)	100 mL (SK3-OT-100)	15-25°C
Oxalic acid, 1% solution	30 mL (OKS1-OT-30)	50 mL (OKS1-OT-50)	100 mL (OKS1-OT-100)	15-25°C
Ammonium iron sulfate, solution	30 mL (ASF-OT-30)	50 mL (ASF-OT-50)	100 mL (ASF-OT-100)	15-25°C
Silver ammonia solution	30 mL (SA-OT-30)	50 mL (SA-OT-50)	100 mL (SA-OT-100)	2-8°C
4% formaldehyde, alcoholic solution	30 mL (F4A-OT-30)	50 mL (F4A-OT-50)	100 mL (F4A-OT-100)	15-25°C
Sodium thiosulfate, 5% solution	30 mL (NT5-OT-30)	50 mL (NT5-OT-50)	100 mL (NT5-OT-100)	15-25°C

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, 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 DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- 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
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade

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.

Sample staining procedure

a) using kit for 100 tests (RE-100T)

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, solution.	5 min
6.	Rinse in distilled (demi) water	until the excessive reagent is washed off of the section
7.	Treat with Oxalic acid, 1% solution (add ≥5 drops)	3 min
8.	Rinse thoroughly in distilled (demi) water twice	until the excessive reagent is washed off of the section
9.	Treat with Ammonium iron sulfate, solution (≥5 drops)	3 min
10.	Rinse thoroughly in distilled (demi) water twice	until the excessive reagent is washed off of the section
11.	Treat with Silver ammonia solution (≥5 drops)	3 min
12.	Rinse in distilled (demi) water	until the excessive reagent is washed off of the section
13.	Treat with 4% formaldehyde, alcoholic solution (≥5 drops)	5 min
14.	Rinse thoroughly in distilled (demi) water twice	until the excessive reagent is washed off of the section
15.	Treat the sections with Sodium thiosulfate, solution (add ≥5 drops),	5 min
16.	Rinse the section in tap water	5 min
17.	Dehydrate using 70% alcohol (Histanol 70)	5 dips

18.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
19.	Dehydrate using 100% alcohol (Histanol 100)	2 min
20.	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.

b) using seven-reagent 50 mL kit (RE-K-50) and 100 mL (RE-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. Prepare the working solution of volume adequate for staining test sections.	
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 Acetic acid, 1% solution	3 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 in Sodium thiosulfate solution	5 min
17.	Rinse the section in tap water	5 min
18.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
19.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
20.	Dehydrate using 100% alcohol (Histanol 100)	2 min
21.	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

Reticular and nerve fibers - dark purple to black

Connective tissue and erythrocytes - brown

Collagen - yellowish-brown

Note

Microbiology staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

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

Components of Reticulin kit are kept under different storage conditions. Keep reagents dry, at temperature indicated on the label in a tightly sealed original packaging. 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		Storage temperature range		Number of tests in package		Product code		European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer
	For in vitro diagnostic use only		Keep in dry place		Caution - fragile				

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