BIOGNOST®

RETICULIN CONTRAST KIT

IVD *In vitro* diagnostic medical device CE

Nine-reagent kit for detecting reticular fibers acc. to Gordon and Sweets
INSTRUCTIONS FOR USE

REF Product code: RET-100T (for 100 tests) RET-K-50 (9x50 mL) RET-K-100 (9x100 mL)

Introduction

Reticulin Contrast 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. Reticulin Contrast kit also contains gold chloride solution that stabilizes and tones the section's image. The kit contains Nuclear Fast Red (Kernechtrot) counterstain.

Product description

RETICULIN CONTRAST KIT - Nine-reagent kit for detecting reticular fibers

The kit contains:	100 tests (RET-100T)	9 x 50 mL (RET-K-50)	9 x 100 mL (RET-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
Gold chloride, 0.2% solution	30 mL (ZK02-OT-30)	50 mL (ZK02-OT-50)	100 mL (ZK02-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
Nuclear Fast Red (Kernechtrot) reagent	30 mL (KR-OT-30)	50 mL (KR-OT-50)	100 mL (KR-OT-100)	15-25°C

CAUTION:

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)

Preparing the histological sections for staining

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

NOTE: Apply the reagent so it completely covers the section.

Sample staining procedure

a) using kit for 100 tests (RET-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, 3% 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)	1 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.	Tone with Gold chloride, 0.2% solution	let it set for 2 min
16.	Rinse in distilled water	
17.	Treat the sections with Sodium thiosulfate, 5% solution (add ≥5 drops),	let it set for 2 min
18.	Rinse in distilled water	

19.	Stain with Nuclear Fast Red (Kernechtrot) reagent (add ≥5 drops)	let it set for 5 min
20.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
21.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
22.	Dehydrate using 100% alcohol (Histanol 100)	2 min
23.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 5 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 nine-reagent 50 mL kit (RET-K-50) and 100 mL (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 ovlumes 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 Acetic 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, 5 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 fibres - dark purple to black, nuclei - pink to red
Collagen – brown black, background – light pink

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.

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 Contrast 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

RET-X, V11-EN7, 06 March 2019, IŠP/VR

	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	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						