BIOGNOST®

RETICULIN CONTRAST KIT

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Nine-reagent kit for detecting reticulin fibers acc. to Gordon and Sweet

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI number**
RET-100T	100 tests	03858890004229
RET-K-50	9x50 mL	03858890005134
RET-K-100	9x100 mL	03858890005141

Intended use and test principle

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 slide'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

Additional reagents and materials that can be used in this method

- 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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade

Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 μm thin slices and mount on a VitroGnost microscope slide

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 a sediment appears in Ammonium iron sulfate, solution reagent, it needs to be filtered (the sediment does not affect staining quality)

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 in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 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 water	until 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 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 excessive reagent is washed off of the section
11.	Treat with Silver ammonia solution (≥5 drops)	3 min
12.	Rinse in distilled water	until 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 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 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 in 70% alcohol (Histanol 70)	5 dips
21.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
22.	Dehydrate in 100% alcohol (Histanol 100)	2 min
23.	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.

b) using nine-reagent kit 50 or 100 mL (RET-K-50, 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 in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized 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 slide into working solution and let it react	5 min
7.	Rinse in distilled/demineralized water	until excessive reagent is washed off of the section
8.	Immerse into Oxalic acid, 1% solution	1 min
9.	Rinse thoroughly in distilled/demineralized water twice	until excessive reagent is washed off of the section
10.	Immerse into Ammonium iron sulfate, solution	3 min
11.	Rinse thoroughly in distilled/demineralized water twice	until excessive reagent is washed off of the section
12.	Immerse into Silver ammonia solution	3 min
13.	Rinse in distilled/demineralized water	until excessive reagent is washed off of the section
14.	Immerse into 4% formaldehyde, alcoholic solution	5 min
15.	Rinse thoroughly in distilled/demineralized water twice	until excessive reagent is washed off of the section
16.	Immerse into Gold chloride, 0.2% solution	2 min
17.	Rinse in distilled/demineralized water	until excessive reagent is washed off of the section
18.	Immerse into Sodium thiosulfate, 5% solution	2 min
19.	Rinse in distilled/demineralized water	until excessive reagent is washed off of the section
20.	Immerse into Nuclear Fast Red (Kernechtrot) reagent	5 min
21.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
22.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
23.	Dehydrate in 100% alcohol (Histanol 100)	2 min
24.	Clear the slide 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

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control.
If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

Storage, stability, and shelf life

Reticulin Contrast kit reagents each have their own storage temperature demands, printed on their respective labels. Keep reagents at their required storage temperatures, in a dry place, do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product 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

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapor.
H317	May cause an allergic skin reaction.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H412	Harmful to aquatic life with long lasting effects.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P273	Avoid release to the environment.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P501	Dispose of contents/container according to local regulations.
P302+P352	IF ON SKIN: wash with plenty of water
P308+P311	IF exposed or concerned: Call a POISON CENTER/doctor/…
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.

RET-IFU_ENV9, 06.02.2026., IŠP

	Manufacturer	LOT	Batch code		Consult instructions for use	Σ	Contains sufficient for <n>tests		Contains hazardous substances
	Date of manufacture	REF	Catalogue number		Caution	CE	European conformity		Fragile, handle with care
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device	UDI	Unique device identifier		

BioGnost Ltd.
Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

Version	Description / reason for change	Date
9	Revised in acc. to Regulation (EU) 2017/746 - IVDR	06.02.2026.