BIOGNOST®

RHODANINE KIT

CE IVD *In vitro* diagnostic medical device Classification according to Regulation (EU) 2017/746 - **Class A** device

Four-reagent kit for detecting copper

INSTRUCTIONS FOR USE

Basic UDI-DI	385889212HPC30708STARVF	
EMDN code	W01030708	
REF **Catalog number**	**Volume**	**UDI-DI**
ROD-100T	For 100 tests	03858890002492
ROD-K-100	5x100 mL	03858890005394

Intended use and test principle

Histology, cytology, and other related scientific disciplines study the microscopic anatomy of tissues and cells. Proper staining is required to achieve good visualization of tissue and cellular structures. The Rhodanine Kit is used for the detection of copper by means of Rhodanine dye in histological samples of liver tissue. The method is based on the selective reaction of 5-(4-dimethylaminobenzylidene) Rhodanine with protein-bound copper, resulting in a red to red-orange stained complex that enables microscopic identification of copper deposits. The staining is used as an aid in the diagnostic workup of copper metabolism disorders, including Wilson's disease and other conditions associated with copper accumulation in tissue, and the results must be interpreted in correlation with clinical, laboratory, and other histopathological findings.

Product description

- **RHODANINE KIT** – Four-reagent kit for copper detection in histological samples

The kit contains:	**ROD-100T (for 100 tests)**	**ROD-K-100 (5 x 100 mL)**	**Storage temperature**
Sodium acetate, solution	30 mL (NA-OT-30)	100 mL (NA-OT-100)	15-25°C
Formaldehyde NB 4%	30 mL (FNB4-30)	100 mL (FNB4-100)	15-25°C
Rhodanine reagent	2 x 30 mL (RR-OT-30)	2 x 100 mL (RR-OT-100)	15-25°C
Hematoxylin M	30 mL (HEMM-OT-30)	100 mL (HEMM-OT-100)	15-25°C

Additional reagents and materials that can be used in the method:

- Fixatives, such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- A clearing agent such as BioClear xylene or a substitute such as BioClear New, an aliphatic hydrocarbon-based agent
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil 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 micron thin slices and mount on a VitroGnost microscope slide

NOTE

Apply the reagent so that it completely covers the section.
To prevent the sections from drying out during the staining procedure, use an incubation tray or box.

Staining procedure for histological sections

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

1.	Deparaffinize in xylene (BioClear) or 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 the buffer solution for rinsing: 40 mL distilled/demineralized water + 10 drops of Sodium acetate, solution + 10 drops of Formaldehyde NB 4%	
6.	Prepare the Rhodanine working solution: mix 20 drops of Rhodanine reagent (shake before use!) in 40 mL of distilled/demineralized water	
	Note: discard the Rhodanine working solution after use	
7.	Immerse the slide in the Rhodanine working solution	20 hr at +37 °C
8.	Wash the slide in the buffer solution for rinsing (prepared in step 5)	
9.	Apply Hematoxylin M to the slide (≥5 drops) or immerse the slide in Hematoxylin M	2 min
	Note: to reduce counterstaining, the incubation time in Hematoxylin M may be less than 2 minutes	1-2 min
10.	Rinse the slide in the buffer solution for rinsing (prepared in step 5)	3 exchanges, 1 min each
11.	Briefly dehydrate through 95% and 100% alcohol (Histanol 95 and Histanol 100)	
12.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

b) using three-reagent kit of 100 mL (ROD-K-100)

Pour the reagents into glass staining jars (type Coplin, Hellendahl, or Schifferdecker) and return them to the original bottles after staining. Close well. Filter the reagents as needed.

1.	Deparaffinize in xylene (BioClear) or 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 the buffer solution for rinsing: 40 mL distilled/demineralized water + 10 drops of Sodium acetate, solution + 10 drops of Formaldehyde NB 4%	
6.	Prepare the Rhodanine working solution: mix 20 drops of Rhodanine reagent (shake before use!) in 40 mL of distilled/demineralized water	
	Note: discard the Rhodanine working solution after use	
7.	Immerse the slide in the Rhodanine working solution	20 hr at 37 °C
8.	Wash the slide in the buffer solution for rinsing (prepared in step 5)	
9.	Immerse the slide in Hematoxylin M	2 min
	Note: to reduce counterstaining, the incubation time in Hematoxylin M may be less than 2 minutes	1-2 min
10.	Rinse the slide in the buffer solution for rinsing (prepared in step 5)	3 exchanges, 1 min each
11.	Briefly dehydrate through 95% and 100% alcohol (Histanol 95 and Histanol 100)	
12.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

Result
Copper - brown-red
Nuclei - blue-purple color

Limitations
This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instructions 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. It is necessary to follow the manufacturer's instructions for use. To avoid errors, the staining process and diagnosis can only be carried out by authorized and professionally qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards. To avoid a false result, it is recommended to use a positive and negative control.
If a serious incident occurs during use or as a result of its use, please report it to the manufacturer and/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, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use and in the safety data sheet, which is available on request.

Storage, stability, and shelf life
Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. 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 production date and expiration date are printed on the product label.

References

1. Culling, C.F.A. (1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
2. Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p. 14-167.

Warnings and precautions regarding the materials contained in the product:

H302	Harmful if swallowed.
H312	Harmful in contact with skin.
H317	May cause an allergic skin reaction.
H332	Harmful if inhaled.
H341	Suspected of causing genetic damage.
H350	May cause cancer.
H225	Highly flammable liquid and vapour.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P310	IF SWALLOWED: Immediately call a POISON CENTER/doctor.
P302+P352	IF ON SKIN: wash with plenty of water.
P304+P340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P308+P313	IF exposed or concerned: Get medical advice/attention.

ROD-IFU_ENV7, 01.07.2026. IŠP

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

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Version	Description / reason for change	Date
7	Revised acc. to Regulation (EU) 2017/746 - IVDR	01.07.2026.