RHODANINE KIT

IVD In vitro diagnostic medical device CE

Four-reagent kit for detecting copper
INSTRUCTIONS FOR USE

REF Catalog number: ROD-100T (for 100 tests) ROD-K-100 (5 x 100 mL)

Introduction

Rhodanine kit is used for detecting copper using Rhodanine in histological samples of liver tissue.

Product description

- **RHODANINE KIT** – Four-reagent kit in five packages for detecting copper in histological samples

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

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:

Apply the reagents so they completely cover the section.

In order to avoid sections getting dried during staining procedure, use a container or incubation box.

Preparing 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 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

Histological sections staining procedure

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Start rehydration by 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 the buffer solution for rinsing: 40 mL of distilled (demi) water + 10 drops of Sodium acetate, solution + 10 drops of 4% NB Formaldehyde	
6.	Prepare Rhodanine working solution: Mix 20 drops of Rhodanine reagent with 40 mL of distilled water	
	Note: discard Rhodanine working solution after use	
7.	Dip the sections in Rhodanine working solution (preparation described in the previous step)	20 hours at 37 °C
8.	Wash the sections in buffer solution for rinsing (preparation described in step 5)	
9.	Add Hematoxylin M to the section (≥5 kapi) or immerse the section into Hematoxylin M	2 min
	Note: in order to reduce the amount of counterstaining, incubation period in Hematoxylin M may be under 2 min	1-2 minutes
10.	Wash the sections in buffer solution for rinsing (preparation described in step 5)	3 exchanges, 1 min each
11.	Quickly dehydrate through 96% and 100% alcohol (Histanol 96 and Histanol 100)	
12.	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.

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.

Result

Copper - brown-red
Nuclei - blue-purple

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 taken care of as a 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

Store Rhodanine kit in a tightly closed original packaging at temperature stated on the product label. In order to prolong Rhodanine reagent's shelf life, we suggest keep it at 2-8°C after first use. Do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's 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.

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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						