

# RHODANINE powder dye

IVD In vitro diagnostic medical device

CE

## 5-(4-Dimethylaminobenzylidene)rhodanine For staining deposits of copper bound to proteins

### INSTRUCTIONS FOR USE

REF Catalog number: RHO-P-5 (5 g)

#### Introduction

5-(4-Dimethylaminobenzylidene)rhodanine is a dye in crystal form, and it is used for preparation of Rhodanine reagent. Along with the rest of the three reagents, Rhodanine reagent is the basic component of Rhodanine kit, used for detecting excessive accumulation of copper in liver - a consequence of the most important disorder in copper metabolism, Wilson's disease.

#### Product description

- **RHODANINE** – dye for preparing Rhodanine reagent used in Rhodanine kit

#### Example of usage of 5-(4-Dimethylaminobenzylidene)rhodanine powder dye in Rhodanine kit

#### 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
- BioGnost's reagents:
  - Sodium acetate, solution, product code NA-OT-30, NA-OT-100
  - Formaldehyde NB 4%, product code FNB4-OT-30, FNB4-OT-100
  - Hematoxylin M, product code HEMM-OT-30, HEMM-OT-100

#### Preparation of Rhodanine reagent

- Dissolve 2 g of 5-(4-Dimethylaminobenzylidene)rhodanine powder dye in 1000 ml of denatured absolute alcohol. The solution is stable for one year at room temperature.

#### 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  $\mu$ m 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 ( $\geq 5$ kapi) or immerse the section into Hematoxylin M Note: in order to reduce the amount of counterstaining, incubation period in Hematoxylin M may be under 2 min	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.

## Result

Copper - brown-red

Nuclei - blue-purple

## Note

The mentioned formulation is only one of the ways of preparing the dye solution. Depending on personal requests and standard laboratory operating procedures, the dye solution can be prepared 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. In order to avoid an erroneous result, a positive and negative check is advised before application.

## 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

Keep Rhodanine powder dye in a tightly closed original package at temperature between 15°C and 25°C. Keep in dry places, do not freeze and avoid exposure to direct sunlight. Expiry date is stated on the product's label.

## References

1. Bancroft, J.D. (2008): Theory and Practice of Histological Techniques. 6<sup>th</sup> edition, CHURCHILL LIVINGSTONE ELSEVIER
2. Carson, F. L. (2007): Histotechnology, A Self-Instructional Text. 2<sup>nd</sup> edition, ASCP

RHO-P-5, V2-EN1, 15 February 2017, AK/VR

	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		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 number		Manufacturer		
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile						