

Rhodanine Stain for Copper - Technical Memo

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| SOLUTION: | 250 ml | 500 ml |
| Rhodanine Stock Stain 0.2%, Alcoholic | Part 10531A | Part 10531B |

Additionally Needed, Rhodanine Stain For Copper:

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| Copper, Animal Control Slides | Part 4130 |
| Hematoxylin Stain, Mayer Modified | Part 1202 |
| Sodium Borate 0.5%, Aqueous | Part 13824 |
| Xylene, ACS | Part 1445 |
| Alcohol, Ethyl Denatured, 100% | Part 10841 |
| Alcohol, Ethyl Denatured, 95% | Part 10842 |
| Coplin Jar, Plastic | Part 5184 (for microwave modification) |

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Rhodanine Stain for Copper, with included microwave modification, is used for the detection of copper and copper-associated protein (CAP) in tissue sections. Abnormal copper accumulations are predominantly found in liver tissue, most notably in Wilson's disease.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below.

PRESTAINING PREPARATION:

1. Prepare Working Rhodanine Solution; combine and mix well.
 - a. *Shake Solution A: Rhodanine Stock Stain 0.2%, Alcoholic well before each use.*
 - b. *Solution A: Rhodanine Stock Stain 0.2%, Alcoholic 3 ml*
 - c. *Distilled Water 47 ml*

STAINING PROCEDURE:

2. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. *See Procedure Notes #1 and #2.*
3. Stain in Working Rhodanine Solution at 60°C for 1-2 hours or at 37°C for 18 hours.

Microwave Modification: See Procedure Note #3.

 - a. *Place slides in a plastic Coplin jar containing Working Rhodanine Solution and microwave for 6 minutes at 70°C.*
4. At the end of incubation (for both oven and microwave), to avoid unwanted slide precipitate, pour off warm Working Rhodanine Solution into a second Coplin jar; reserve and set aside.
5. Rinse slides well in several changes of distilled water.
6. Check positive control slide microscopically to determine adequate copper/reddish brown development.
 - a. *Return slides to reserved Working Rhodanine Solution if additional incubation is required.*
7. Prepare dilute Mayer Hematoxylin Stain Solution directly before use; combine and mix well:
 - a. *Hematoxylin Stain, Mayer Modified (1202) 20 ml*
 - b. *Distilled Water 20 ml*

8. Stain in dilute Mayer Hematoxylin Stain Solution for 10 minutes.
9. Rinse in distilled water.
10. Rinse in Sodium Borate 0.5%, Aqueous (13824); 2-3 quick dips.
11. Rinse well in distilled water.
12. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Copper Copper/reddish brown
 Nuclei Light blue

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 251.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 258-260
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 230.
4. Modifications developed by Newcomer Supply Laboratory.