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Part 10531
Revised April 2025

Rhodanine Stain for Copper - Technical Memo

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

Additionally Needed, Rhodanine Stain For Copper:

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 for the detection of copper and copper-associated protein (CAP). Abnormal copper accumulations are predominantly found in liver tissue, most notably Wilson's disease.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (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 provided staining procedure.

PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. Prepare Working Rhodanine Solution; combine and mix well.
   a. *Shake Rhodanine Stock Stain 0.2%, Alcoholic well before each use.*
   b. *Rhodanine Stock Stain 0.2%, Alcoholic 3 ml*
   c. *Distilled Water 47 ml*

STAINING PROCEDURE:

3. 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.*
4. Stain in Working Rhodanine Solution (Step #1) 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. Microwave for 6 minutes at 70°C.*
5. 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.
6. Rinse slides well in several changes of distilled water.
7. 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.*
8. Prepare dilute Mayer Hematoxylin Stain directly before use; combine and mix well:
   a. *Hematoxylin Stain, Mayer Modified (1202) 20 ml*
   b. *Distilled Water 20 ml*
9. Stain in dilute Mayer Hematoxylin Stain Solution for 10 minutes.
10. Rinse in distilled water.
11. Rinse in Sodium Borate 0.5%, Aqueous (13824); 2-3 quick dips.
12. Rinse well in distilled water.
13. 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 slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. The microwave procedure was tested using a laboratory-grade microwave oven. This procedure is a guideline and techniques should be developed for use in your laboratory.
4. If using a xylene substitute, follow manufacturer's recommendation 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.