

## Copper, Rhodanine Stain Kit - Technical Memo

### KIT INCLUDES:

Solution A: Rhodanine Stock Stain 0.2%, Alcoholic	50 ml
Solution B: Hematoxylin Stain, Mayer Modified	250 ml
Solution C: Sodium Borate 0.5%, Aqueous	500 ml

### Part 9113A

**COMPLIMENTARY POSITIVE CONTROL SLIDES:** Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

*Individual stain solutions and additional control slides may be available for purchase under separate part numbers at [www.newcomersupply.com](http://www.newcomersupply.com).*

### Additionally Needed:

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 bottle labels.**

### APPLICATION:

Newcomer Supply Copper, Rhodanine Stain Kit procedure, 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 Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

### PRESTAINING PREPARATION:

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

### STAINING PROCEDURE:

- 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.
  - See Procedure Notes #1 and #2.
- 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.

  - Place slides in a plastic Coplin jar containing Working Rhodanine Solution and microwave for 6 minutes at 70°C.
- 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.
- Rinse slides well in several changes of distilled water.
- Check positive control slide microscopically to determine adequate copper/reddish brown development.
  - Return slides to reserved Working Rhodanine Solution if additional incubation is required.

- Prepare dilute Mayer Hematoxylin Stain directly before use; combine and mix well.
  - Solution B: Hematoxylin Stain, Mayer Modified 20 ml
  - Distilled Water 20 ml
- Stain in dilute Mayer Hematoxylin Stain for 10 minutes
- Rinse in distilled water.
- Rinse in Solution C: Sodium Borate 0.5%, Aqueous; 2-3 quick dips.
- Rinse well in distilled water.
- 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:

- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- 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.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

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