ScyTek LABORATORIES INC.

Instructions For Use

RSS-IFU

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Rhodanine Solution (Stock)

Description and Principle
Rhodanine Solution (Stock) is used in the Copper Stain Kit to demonstrate copper deposits in tissue sections.

Expected Results

Copper Deposits:	Light Brown to Red
Nuclei:	Blue

Kit Contents (Cat# CSK-1)	**Storage**
Additional Kit Reagents Sold Separately	
1. Rhodanine Solution (Stock)	2-8°C
2. Acetate Buffer Solution, pH 8.0	18-25°C
3. Hematoxylin, Mayer's (Lillie's Mod.)	18-25°C

Suggested Controls *(not provided)*
Fetal Liver or a known positive.

Uses/Limitations
For In-Vitro Diagnostic use only.
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage
Mixed storage conditions. Store according to individual label instructions.

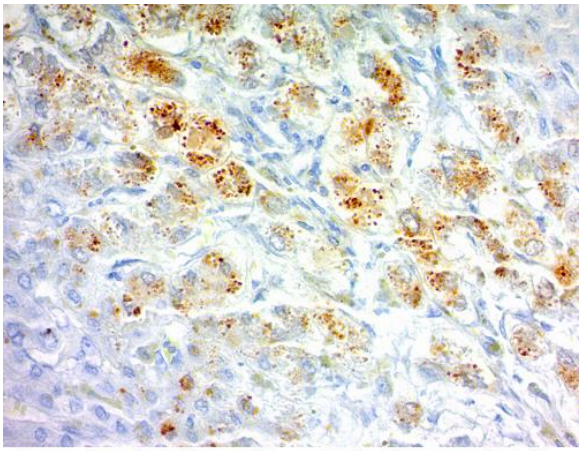
Safety and Precautions
Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

Important Procedural Notes:
1) Rhodanine beings to precipitate immediately once mixed with the Acetate Buffer Solution. This precipitation can reduce the overall staining level. It's important to mix the solutions just before staining and use immediately. Pale yellow mixtures with high precipitation or flocculation may not perform satisfactorily.

2) If copper staining is light or absent, increase the concentration of Rhodanine Stock solution to Acetate buffer and re-run staining.

3) Other methods of heating the "Working Rhodanine Solution" may be used but must be validated by the user.

4) Allow Rhodanine stock solution to come to room temperature and shake well before use.



Copper deposits in Human Liver stained with Copper Stain Kit. Viewed at 400X magnification.

Procedure (Standard)

1. Deparaffinize sections if necessary and hydrate to distilled water.

Prepare **Working Rhodanine Solution** in a clean staining jar by mixing Stock Rhodanine **1:10** with Acetate Buffer Solution. For example, mix 5mls of Stock Rhodanine with 45mls Acetate buffer. Mix well and use immediately, do not store for later use.

2. Place slide(s) in Working Rhodanine Solution and microwave at full power until solution is hot. Do not allow solution to boil.

3. Cap container, gently agitate to mix evenly, and allow solution to cool on countertop to room temperature with occasional agitation.

4. Examine slide microscopically and repeat heating/cooling cycle (steps 3 & 4) until desired staining intensity is achieved.

5. Rinse slide in 2 changes of Acetate Buffer Solution, pH 8.0 for 1 minute each.

6. Rinse briefly with deionized water.

7. Stain tissue section with Hematoxylin, Mayer's (Lillie's Modification) for 5-10 seconds. Increase incubation time for stronger nuclear staining.

8. Rinse briefly with deionized water

9. Rinse slide in Acetate Buffer Solution, pH 8.0 for 1 minute.

10. Dehydrate slide in 3 changes of absolute alcohol.

11. Clear in 2 changes of xylene or xylene substitute, and mount in synthetic resin.

Procedure (Dropper) – Single slide

1. Deparaffinize sections if necessary and hydrate to distilled water.

Prepare **Working Rhodanine Solution** in provided 8ml dropper vial by mixing:

- 1 Drop Rhodanine Solution (Stock).
- 9 Drops Acetate Buffer Solution, pH 8.0.

2. Place a 125ml beaker containing 100ml of water in microwave and heat to nearly boiling.

3. After heating water, carefully lay slide across the top of the beaker containing the hot water and apply 5-10 drops of Working Rhodanine solution. Rising heat and steam from water will warm slide and enhance staining.

4. Allow Working Rhodanine solution to incubate on tissue section until water has cooled to room temperature. Check occasionally to ensure that the tissue section is not allowed to dry.

5. Examine slide microscopically and repeat heating/cooling cycle (steps 2-4) until desired staining intensity is achieved.

6. Rinse slide in 5-10 drops of Acetate Buffer Solution, pH 8.0 for 1 minute, shake off excess and repeat.

7. Rinse briefly with deionized water.

8. Stain tissue section with 5-10 drops of Hematoxylin, Mayer's (Lillie's Modification) for 5-10 seconds. Increase incubation time for stronger nuclear staining.

9. Rinse briefly with deionized water

10. Rinse slide in 5-10 drops of Acetate Buffer Solution, pH 8.0 for 1 minute.

11. Dehydrate slide in 3 changes of absolute alcohol.

12. Clear in 2 changes of xylene or xylene substitute, and mount in synthetic resin.

References

1. Sheehan, DC., Hrapchak, BB. Theory and Practice of Histotechnology; 1980, page 230.
2. Lindquist, RR. Studies on the Pathogenesis of Hepatolenticular II: Cytochemical methods for the location of copper. Arch Pathol; 1969, Volume 87: page 370.

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