

LINDQUIST'S METHOD FOR COPPER (WILSON'S DISEASE) (Microwave)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of copper.
PRINCIPLE:	Rhodanine competes with copper for its binding site thus demonstrating copper associated protein.
CONTROL:	Fetal liver or other known positive for copper <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs029.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	1. Rhodanine Working Solution Item# s2044 2. Gill's Hematoxylin III Item# s211 3. Sodium Borax 0.5% Aqueous Item# s2041 <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	It is essential that specimens destined for staining with this method be fixed in neutral fixative solutions since acid solutions will remove copper. Copper is also diminished after a few months fixation in 10% Buffered Neutral Formalin in the case of fetal liver or liver with very low copper concentrations. Sections which are cut thicker than normal will stain darker. This fact can be used to an advantage when one is having difficulty demonstrating minute amounts of copper.
REFERENCE:	Luna, Lee G. <u>Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts</u> . American Histolabs Inc. Gaithersburg, MD. 1992. pp. 343-344.

STAINING PROCEDURE:

Note: Variations in timing may occur due to the power wattage of the microwave oven. Provided times and power levels are based on 1000 watt microwave oven.

1. Deparaffinize and hydrate slides to distilled water.
2. Place 50 mL of Rhodanine Working Solution in a plastic coplin jar (loosely apply cap) and microwave for 25 seconds. Stir and re-microwave for 25 seconds. Stir and re-microwave for an additional 10 seconds. Place slides in solution for 10 minutes.
3. Wash well in distilled water.
4. Place 50 mL of Gill's Hematoxylin III in a plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave an additional 20 seconds. Place slides in solution for 1 minute.
5. Rinse slides in distilled water.
6. Quickly rinse in Sodium Borax 0.5% Aqueous.
7. Rinse slides in distilled water.
8. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
9. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Copper..... Bright Red
Nuclei..... Blue

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