Diagnostic BioSystems

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Copper Stain Kit (For Microwave)

Catalog Number: KT033

Document #: DS-3006-A
Effective Date: 02/015/15

Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
The Copper Stain Kit (For Microwave) is intended for the demonstration of Copper deposits in tissue sections.

Copper Deposits: Light Brown to Red
Nuclei: Blue

Control Tissue
Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 µm and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Recommended Positive Control
1. Fetal Liver

Reagents Provided

Kit Contents	Volume	Storage
Rhodanine Solution (Stock)	30 mL	2-8°C
Acetate Buffer Solution, pH 8.0	2 X 500 mL	15-30°C
Hematoxylin, Mayer's (Lillie's Mod.)	125 mL	15-30°C

Storage and Handling
Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Prepare the Following Solutions Immediately Before Use
Prepare Working Rhodanine Solution:
1. Combine 4 mL Rhodanine Solution (Stock). Agitate Stock Solution immediately before adding to Acetate Buffer.
2. Add 46 mL Acetate Buffer Solution, pH 8.0.

Staining Procedure
1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place loosely capped staining jar containing Working Rhodanine in microwave and heat solution until warm but not hot.
3. Place slide in warmed Working Rhodanine Solution and microwave at full power until solution is hot. Do not allow solution to boil.
4. Cap container, carefully agitate, and allow solution to cool on countertop to room temperature.
5. Examine slide microscopically and repeat heating/cooling cycle (steps 3 & 4) until desired staining intensity is achieved.
6. Rinse slide in 2 changes of Acetate Buffer Solution, pH 8.0 for 1 minute each.
7. Incubate slide in Hematoxylin, Mayer's (Lillie's Modification) for 5-10 seconds with agitation.
8. Rinse slide in 3 changes of Acetate Buffer Solution, pH 8.0 for 1 minute each.
9. Clear in 2 changes of xylene or xylene substitute, and mount in synthetic resin.

Limitations of the Procedure
1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

Precautions
1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.

Troubleshooting
If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

References

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

Diagnostic BioSystems
6616 Owens Drive
Pleasanton, CA 94588
Tel: (925) 484 3350
www. dbiosys.com

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Emergo Europe
Prinsessegracht 20
2514 AP, The Hague
The Netherlands
Tel: (31) (0) 70 345 8570