Diagnostic BioSystems

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Reticulum Stain Kit

Catalog Number: KT031

**This data sheet is applicable to all sizes (volume) of product. Actual volume is indicated on vial.

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
The Reticulum Stain Kit is intended for use in histological demonstration of reticular fibers. The main function of reticular fibers is to provide support. They are normally found throughout the body, particularly in liver, lymph node, spleen and kidney. Ammoniacal silver stains are the most commonly used methods for demonstration of reticular fibers.

Reticulum: Black
Nuclei: Red

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. Liver
2. Kidney
3. Lymph Node
4. Spleen

Reagents Provided

Kit Contents	Volume	Storage
Potassium Permanganate Solution (1%)	125 mL	15-30°C
Potassium Metabisulfite Solution (3%)	125 mL	15-30°C
Ferric Ammonium Sulfate Solution (3%)	125 mL	15-30°C
Formalin Solution (20%)	125 mL	15-30°C
Gold Chloride Solution (0.1%)	125 mL	2-8°C
Sodium Thiosulfate Solution (5%)	125 mL	15-30°C
Nuclear Fast Red Solution	125 mL	15-30°C
Sodium Hydroxide Solution (3%)	125 mL	15-30°C
Silver Nitrate Solution (10%)	10 ml x 5 vials	2-8°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.

Materials Required but not Provided

1. Concentrated Ammonium Hydroxide Solutions
2. Graded Alcohols
3. Xylene

Prepare the Following Solutions Before Use

1. Prepare working Ammoniacal Silver Solution using chemically cleaned glassware in a chemical fume hood as follows:
2. add and mix 1ml of Concentrated Ammonium hydroxide (25-30%) (Not Included in Kit) and 10ml of Sodium Hydroxide Solution (3%) to the silver solution. The mixture will initially turn brown and then start to become clear.
3. While continuously mixing, carefully add more Concentrated Ammonium hydroxide (25-30%) drop by drop until no precipitate remains and solution is completely clear
4. Avoid adding excess ammonium hydroxide past this point as it may affect staining.
5. Add Distilled Water to a total volume of 60ml and mix completely. Solution is now ready for use.

Note: Use extreme care in preparation and use of Ammoniacal Silver Solution. Store Ammoniacal Silver Solution in a refrigerator to avoid the formation of explosive compounds. If Ammoniacal Silver Solution is exposed to sunlight, it will explode. Dispose of waste observing all local, state and federal laws.

Staining Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place slide in Potassium Permanganate Solution (1%) for 5-10 minutes.
3. Rinse in 3 changes of distilled water.
4. Differentiate in Potassium Metabisulfite Solution (3%) until section is transparent.
5. Rinse in 3 changes of distilled water.
6. Apply Ferric Ammonium Sulfate Solution (3%) for 10 minutes.
7. Rinse in 2 quick changes of distilled water.
8. Apply working Ammoniacal Silver Solution for 2-3 minutes.
9. Rinse in 3 changes of distilled water.
10. Place slide in Formalin Solution (20%) for 1 minute.
11. Rinse in 3 changes of distilled water.
12. Apply Gold Chloride Solution (0.1%) for 3-5 minutes.
13. Rinse in 2 changes of distilled water.
14. Apply Sodium Thiosulfate Solution (5%) for 1-2 minutes to remove unreduced silver.
15. Rinse in tap water for 2 minutes.
16. Counterstain using Nuclear Fast Red Solution for 2-5 minutes.
17. Rinse in tap water followed by distilled water.
18. Dehydrate through 3 changes of Absolute Alcohol.
19. Clear, 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

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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. Use extreme care in preparation and use of Ammoniacal Silver Solution.
2. Store Ammoniacal Silver Solution in a refrigerator to avoid the formation of explosive compounds.
3. If Ammoniacal Silver Solution is exposed to sunlight, it will explode.
4. Consult local and/or state authorities with regard to recommended method of disposal.
5. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
6. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
7. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
8. If reagent contacts these areas, rinse with copious amounts of water.
9. 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. Gomori, G., A Modification of the Silver Impregnation Method of Staining Reticular Fibers. American Journal of Clinical Pathology, Volume 21, Pages 897-899, 1951.