

Instructions For Use ES3403-IFU

Rev. Date: Jan. 24, 2022

Revision: 5

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Reticulum Stain Kit

Description: 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 Background: Red

Uses/Limitations: For In-Vitro Diagnostic use only.

Histological applications.

Do not use past expiration date.

Use caution when handling these reagents.

Control Tissue: Liver

Kidney Lymph Node Spleen

Kit Contents:

Description	<u>Volume</u>	Storage .
Potassium Permanganate Solution (1%)	125 ml	18-25° C.
Potassium Metabisulfite Solution (3%)	125 ml	18-25° C.
Ferric Ammonium Sulfate Solution (3%)	125 ml	18-25° C.
Formalin Solution (20%)	125 ml	18-25° C.
Gold Chloride Solution (0.1%)	125 ml	2-8° C.
Sodium Thiosulfate Solution (5%)	125 ml	18-25° C.
Nuclear Fast Red, Sodium Hydroxide 3%	125 ml	18-25° C.
Sodium Hydroxide Solution (3%)	125 ml	18-25° C.
Silver Nitrate Solution (10%)	10 ml x 5 vials	2-8° C.

Required but not included:

Ammonium Hydroxide Solution, Concentrated

Graded Alcohols

Xylene

Precautions: Keep away from open flame.

Avoid contact with skin and eyes.

Harmful if swallowed.

Follow all Federal, State, and local regulations regarding disposal.

Use in chemical fume hood whenever possible.

Wear protective clothing.

Storage: 2° C 25° C

Mixed Storage Conditions. Separate Contents.



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Preparation of Reagents Prior to Beginning:

1. Prepare working Ammoniacal Silver Solution using chemically cleaned glassware in a chemical fume hood as follows: To 17ml of Distilled Water add one (10ml) vial of Silver Nitrate Solution (10%) and mix completely. Add 1ml of Concentrated Ammonium Hydroxide (Not Included in Kit) while continuously mixing. The mixture will initially turn brown and then become clear. Add 10ml of Sodium Hydroxide Solution (3%) and mix completely. If the solution does not remain colorless, add Concentrated Ammonium Hydroxide drop by drop until no precipitate remains. 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.

Procedure (Standard):

- 1. Deparaffinize sections if necessary and hydrate to distilled water.
- 2. Place slide in Potassium Permanganate Solution (1%) for 5-10 minutes.
- Rinse in 3 changes of distilled water.
- 4. Differentiate in Potassium Metabisulfite Solution (3%) until section is transparent.
- 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.
- 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, Sodium Hydroxide 3% for 2-5 minutes.
- 17. Rinse briefly with Absolute Alcohol.
- 18. Dehydrate through 3 changes of Absolute Alcohol.
- 19. Clear, and mount in synthetic resin.

References:

1. Gomori, G., A Modification of the Silver Impregnation Method of Staining Reticular Fibers. American Journal of Clinical Pathology, Volume 21, Pages 897-899, 1951.

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