

SNOOK'S METHOD FOR RETICULUM

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of reticulum.
PRINCIPLE:	Potassium Permanganate first oxidizes the reticulum fibers to produce active sites which readily take up the silver from the Diamino Silver Solution. Formaldehyde then reduces the silver to the metallic form and Gold is used to tone the stain. Finally, Sodium Thiosulfate removes unreacted silver and a counterstain is used if desired.
CONTROL:	Liver <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs024.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	1. Potassium Permanganate 0.25% Aqueous Item# s2273 2. Oxalic Acid 5% Aqueous Item# s2199 3. Uranyl Nitrate 1% Aqueous Item# s288B 4. Formalin 1% Non Buffered Item# s2000 5. Gold Chloride 1% Aqueous Item# s203 6. Sodium Thiosulfate 5% Aqueous Item# s1895 7. Nuclear Fast Red Kernechtrot 0.1% Item# s248 8. Silver Nitrate 5% Aqueous Item# s1890 9. Sodium Hydroxide 10% Aqueous Item# s1922 10. Ammonium Hydroxide Concentrated Item# c804 <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	
REFERENCE:	Luna, Lee G. <u>Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology</u> . 3rd Ed. McGraw-Hill Book Co. New York. 1968. pp. 90-91.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Oxidize in Potassium Permanganate 0.25% Aqueous for 5 minutes.
3. Wash in tap water.
4. Place in Oxalic Acid 5% Aqueous for 5 minutes.
5. Wash in water.
6. Mordant in Uranyl Nitrate 1% Aqueous for 1 minute.
7. Wash in running water.
8. Place in freshly made Ammoniacal Silver Solution for 1 minute.
Directions for Ammoniacal Silver Solution:
To 20 mL of Silver Nitrate 5% Aqueous in an acid cleaned graduated glass cylinder, add 20 drops of Sodium Hydroxide 10% Aqueous. While shaking cylinder, add Ammonium Hydroxide Concentrated drop by drop, until only a few granules of the resulting precipitate remain on the bottom of the cylinder. Add distilled water to make 60 mL. Filter and use immediately.
9. Dip in running water.
10. Place in Formalin 1% Non Buffered for 1 minute.
11. Wash in running water.
12. Place in Gold Chloride 1% Aqueous until sections are grayish black (1 minute).
13. Wash in running water.
14. Place in Sodium Thiosulfate 5% Aqueous for 30 seconds to 1 minute.
15. Wash in running water.
16. Counterstain (if desired) in Nuclear Fast Red Kernechtrot 0.1% for 3 minutes.
17. Rinse in distilled water.
18. Dehydrate in Alcohol and clear in Xylene, 2 changes each.
19. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Reticulum Fibers..... Gray to Black
Background Pink to Rose

Poly Scientific R&D Corp.

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