REAGENT ASSEMBLY KIT
ITEM# k082

GORDON SWEETS' RETICULUM METHOD

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of reticulum fibers.
PRINCIPLE:	Potassium Permanganate first oxidizes the reticular fibers to produce active sites which readily take up the silver from the Diamino Silver Solution. Formaldehyde then reduces the silver to the visible metallic form and Gold is used to tone the stain. Finally, a counterstain is used if desired.
CONTROL:	Normal liver *Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs024.*
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 5 micrometers
SOLUTIONS:	1. Sulfuric Acid 3% Aqueous Item# s2363 2. Potassium Permanganate 0.5% Aqueous Item# s263 Acidified Potassium Permanganate Working Solution: Must be prepared fresh each time. Sulfuric Acid 3% Aqueous ..... 5 mL Potassium Permanganate 0.5% Aqueous ..... 95 mL 3. Oxalic Acid 1% Aqueous Item# s2328 4. Formaldehyde 37% USP Item# c808 5. Gold Chloride 0.5% Aqueous Item#s2364 6. Neutral Red 1% Aqueous Item# s2113 7. Ferric Ammonium Sulfate 2.5% Aqueous Item# s2253 8. Silver Nitrate 10% Aqueous Item# s1891 9. Sodium Hydroxide 3% Aqueous Item# s1920 10. Ammonium Hydroxide Concentrated Item# c804 Silver Nitrate Working Solution: Prepare fresh. Place 5 mL of Silver Nitrate 10% Aqueous in a graduated glass cylinder. Add Ammonium Hydroxide drop by drop. Shake solution continuously. Precipitate will form and then will turn black. Add Ammonium Hydroxide (approximately 17 drops) until solution turns light smoky color. Add by pipette, 5 mL of Sodium Hydroxide 3% Aqueous. A new black precipitate will form. Add Ammonium Hydroxide drop by drop until solution is slightly opaque (14 drops). Add distilled water until total volume is 80 mL. *Solutions can be purchased separately from Poly Scientific.*
NOTES:	
REFERENCE:	Luna, Lee G. Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts. American Histolabs Inc. Gaithersburg, MD. 1992. pp. 446-448.

STAINING PROCEDURE:

1. Deparaffinize and hydrate sections to distilled water.
2. Oxidize in Acidified Potassium Permanganate Working Solution, 5 minutes.
3. Wash well in water, 3 minutes.
4. Bleach in Oxalic Acid 1% Aqueous until there is no trace of brown color.
5. Rinse well in tap water, 5 minutes.
6. Place in Ferric Ammonium Sulfate 2.5% Aqueous, 15 minutes. It is important that no crystals be at the bottom of staining dish. If so, fresh solution must be made.
7. Rinse slides in distilled water, several changes. Leave slides in distilled water until staining jars have been prepared in the following order:
   a. Silver Nitrate Working Solution, 1 jar b. Distilled water, 3 jars
   c. Formaldehyde 37%, 1 jar d. Distilled water, 3 jars
8. Place in Silver Nitrate Working Solution, 10 dips or more.
9. Rinse slide well in distilled water, 3 changes, 15 dips each. Water must be changed after each slide.
10. Place slide in Formaldehyde 37%, 3 dips. The reaction takes place immediately. Do not leave slide in this solution.
11. Rinse well in distilled water, 3 changes, 10 dips each. Check under microscope. The fibers should be dark black and the background colorless or at most, yellowish-brown. If the reticulum fibers are not black, the slide should be rinsed at least 3 minutes in running water, and the procedure repeated from step 8. The slide must be rinsed well. If this is not done, when returned from the Formaldehyde to the Silver stain, the remaining trace of Formaldehyde will reduce the Silver Nitrate Working Solution and render it useless. If fibers are black, rinse in 3 changes of distilled water.
12. Tone slide in Gold Chloride 0.5% Aqueous 1-12 dips. Do not overdo. If the slide stays too long in Gold Chloride, it will turn a reddish color.
13. Rinse well in water, 5 minutes.
14. Counterstain if desired in Neutral Red 1% Aqueous.
15. Rinse in water, 15 dips.
16. Dehydrate, clear, and mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Reticulum Fibers ..... Black

Background ..... Red