

## Silver Nitrate 10%, Aqueous for Fontana Masson - Technical Memo

### SOLUTION:

Silver Nitrate 10%, Aqueous	250 ml Part 13806A	500 ml Part 13806B
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### Additionally Needed:

Melanin Control Slides	Part 4430	or	Argentaffin Control Slides	Part 4035
Ammonium Hydroxide 28-30%, ACS	Part 1006			
Gold Chloride 0.2%, Aqueous	Part 11286			
Sodium Thiosulfate 5%, Aqueous	Part 1389			
Nuclear Fast Red Stain, Kernechtrot	Part 1255			
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)			

**For storage requirements and expiration date refer to individual product labels.**

### APPLICATION:

Newcomer Supply Fontana Masson Stain procedure demonstrates argentaffin substances such as melanin, argentaffin granules of carcinoid tumors, and some neurosecretory granules. This technique is not specific for melanin and argentaffin and other reducing substances, such as formalin pigment, will also give a positive reaction.

### METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (1090)

**Technique:** Paraffin sections cut at 4 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

### PRESTAINING PREPARATION:

- If necessary, heat dry sections/slides in oven.
- All glassware/plasticware must be acid cleaned prior to use.
  - See Procedure Notes #1 and #2.
- Prepare Fontana Working Solution (diamine silver) in an acid cleaned Erlenmeyer flask:
  - Silver Nitrate 10%, Aqueous; 25 ml
  - Add Ammonium Hydroxide 28-30%, ACS (1006) drop by drop, mix with swirling motion until solution clouds, then clears. Use caution to not add excess Ammonium Hydroxide.
  - Add more Silver Nitrate 10%, Aqueous drop by drop until clear solution becomes slightly cloudy.
  - Let solution stand 2-4 hours before use.**
  - For use; after standing, filter silver solution. Combine 20 ml of filtered diamine silver solution with 40 ml of distilled water; 60 ml total.

### STAINING PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
  - See Procedure Notes #3 and #4.
- Immerse in Fontana Silver Working Solution (Step #3) in 45° - 60°C water bath for 1 hour.
- Check slides microscopically; remove control, rinse in warm distilled water. Confirm reaction is complete when granules are dark brown and background is colorless.
  - Return to heated Fontana Silver Working Solution for longer incubation if indicated.

- Rinse well in three changes of distilled water.
- Immerse in Gold Chloride 0.2%, Aqueous (11286); 10 minutes.
- Rinse well in distilled water.
- Place in Sodium Thiosulfate 5%, Aqueous (1389); 5 minutes.
- Rinse well in distilled water.
- Counterstain in Nuclear Fast Red Stain, Kernechtrot (1255) for 5 minutes.
  - Shake solution well before use; do not filter.
- Rinse well in distilled water.
  - See Procedure Note #5.
- Dehydrate quickly in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

### RESULTS:

Melanin and argentaffin granules	Black
Nuclei	Pink-red

### PROCEDURE NOTES:

- Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water.
- No metals of any kind should come in contact with silver solutions to prevent precipitation of silver salts. Use plastic forceps (5500) or paraffin coated metal forceps.
- Drain slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during procedure.
- Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
- If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

### REFERENCES:

- Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 286-287.
- Sheehan, Dezna C. and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 276-277.
- Modifications developed by Newcomer Supply Laboratory.

## Silver Nitrate 10%, Aqueous for Reticulum, Gordon & Sweets - Technical Memo

<b>SOLUTION:</b>	<b>250 ml</b>	<b>500 ml</b>	
Silver Nitrate 10%, Aqueous	Part 13806A	Part 13806B	
<b>Additionally Needed:</b>			
Ammonium Hydroxide 28-30%, ACS	Part 1006	Reticulum Control Slides	Part 4620
Potassium Permanganate 1%, Aqueous	Part 13393		
Oxalic Acid 5%, Aqueous	Part 1293		
Ferric Ammonium Sulfate 2.5%, Aqueous			
Sodium Hydroxide 3%, Aqueous			
Formalin 10%, Phosphate Buffered	Part 1090		
Gold Chloride 0.2%, Aqueous	Part 11286		
Sodium Thiosulfate 5%, Aqueous	Part 1389		
Nuclear Fast Red Stain, Kernechtrot	Part 1255		
Xylene, ACS	Part 1445		
Alcohol, Ethyl Denatured, 100%	Part 10841		
Alcohol, Ethyl Denatured, 95%	Part 10842		
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)		

**For storage requirements and expiration date refer to individual product labels.**

### APPLICATION:

Newcomer Supply Reticulum, Gordon & Sweets Stain procedure is a silver staining method for demonstration of reticular fibers; regarded as specialized connective tissue fibers.

### METHOD:

**Fixation:** Formalin 10%, Phosphate Buffered (1090)

**Technique:** Paraffin sections cut at 4 microns

**Solutions:** All solutions manufactured by Newcomer Supply, Inc.

### PRESTAINING PREPARATION:

1. If necessary, heat dry tissue sections/slides in oven.
2. All glassware/plasticware must be acid cleaned prior to use.
  - a. See Procedure Notes #1 and #2.
3. Prepare Ammoniacal Silver Working Solution. Save for Step #11.
  - a. Place 5 ml Silver Nitrate 10%, Aqueous in a flask.
  - b. Add Ammonium Hydroxide 28-30%, ACS (1006) drop by drop, continuously swirling until precipitate completely dissolves. Do not add any excess Ammonium Hydroxide.
  - c. Add 5 ml of Sodium Hydroxide 3%, Aqueous.
  - d. Re-dissolve precipitate with Ammonium Hydroxide 28-30%, ACS drop by drop, until a faint cloudiness remains.
  - e. If proceeded too far and no cloudiness remains, add Silver Nitrate 10%, Aqueous drop by drop, until one drop causes solution to become permanently cloudy. Faint cloudiness is optimum.
  - f. Bring solution volume to 50 ml with distilled water; filter.

### STAINING PROCEDURE:

4. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
  - a. See Procedure Notes #3 and #4.
5. Oxidize in Potassium Permanganate 1%, Aqueous (13393) for 3 minutes.
6. Wash in running tap water for 1 minute; rinse in distilled water.
7. Bleach in Oxalic Acid 1%, Aqueous for 2 minutes or until sections are colorless.
  - a. Oxalic Acid 5% Aqueous (1293) 10 ml
  - b. Distilled water 40 ml
8. Wash in running tap water for 1 minute; rinse in distilled water.
9. Sensitize in Ferric Ammonium Sulfate 2.5%, Aqueous; 10 to 15 minutes.
10. Rinse in several changes of distilled water.
11. Impregnate sections in filtered Ammoniacal Silver Working Solution (Step #3); 2 minutes.

12. Rinse well in running distilled water for 1 minute.
13. Reduce in Formalin 10%, Phosphate Buffered (1090) for 1 minute.
14. Rinse in running tap water for 3 minutes.
15. Check control microscopically for black reticular fiber development.
  - a. See Procedure Note #5.
16. Tone in Gold Chloride 0.2%, Aqueous (11286) for 1-2 minutes.
17. Rinse well in distilled water.
18. Place in Sodium Thiosulfate 5%, Aqueous (1389) for 1 minute.
19. Wash well in tap water for 1 minute; rinse in distilled water.
20. Counterstain with Nuclear Fast Red Stain, Kernechtrot (1255) for 5 minutes.
  - a. Shake solution well before use; do not filter.
21. Rinse well in distilled water.
  - a. See Procedure Note #6.
22. Quickly dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

### RESULTS:

Reticular fibers	Black	Background	Red
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### PROCEDURE NOTES:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water.
2. No metals of any kind should come in contact with silver solutions to prevent precipitation of silver salts. Use plastic forceps (5500) or paraffin coated metal forceps.
3. Drain slides after each step to avoid solution carry over.
4. Do not allow sections to dry out at any point during procedure.
5. If black reticular fibers are not evident or are lightly/poorly stained, return all slides to Silver Working Solution (Step #11) and repeat Steps 11-14 with the same timings.
6. Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
7. If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

### REFERENCES:

1. Carson, Freida L. and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 177-179.
2. Gordon, Harold and Henry Sweets. "A Simple Method for the Silver Impregnation of Reticulum." *American Journal of Pathology* 12.4 (1936): 545-552.
3. Modifications developed by Newcomer Supply Laboratory.