

Fontana Masson Stain - Technical Memo

SOLUTIONS:	100 ml	250 ml	500 ml	1 Liter
Silver Nitrate 10%, Aqueous		Part 13806A	Part 13806B	
Ammonium Hydroxide 28-30%, ACS			Part 1006A	
Gold Chloride 0.2%, Aqueous	Part 11286A	Part 11286B		
Sodium Thiosulfate 5%, Aqueous			Part 1389A	Part 1389B
Nuclear Fast Red Stain, Kernechtrot		Part 1255A	Part 1255C	Part 1255B

Additionally Needed:

Melanin Control Slides	Part 4430	or	Argentaffin Control Slides	Part 4035
Hydrochloric Acid 5%, Aqueous	Part 12086 (for acid cleaning glassware)			
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			

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

APPLICATION:

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

METHOD:

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

Technique: Paraffin sections cut at 5 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 staining procedure provided below.

STAINING PROCEDURE:

- All glassware/plasticware must be acid cleaned prior to use.
 - See Procedure Notes #1 and #2.
- Prepare Fontana Silver Working Solution (diamine silver) in an acid cleaned Erlenmeyer flask:
 - Silver Nitrate 10%, Aqueous 25 ml
 - Add Ammonium Hydroxide 28-30%, ACS drop by drop, mixing with swirling motion until solution clouds, then clears. Use caution to not add too much Ammonium Hydroxide 28-30%, ACS.
 - Add more Silver Nitrate 10%, Aqueous drop by drop until clear solution becomes slightly cloudy.
 - Let solution stand for 2-4 hours before use.**
 - For use; after standing, filter the solution. Then combine 20 ml of this filtered diamine silver solution with 40 ml of distilled water; 60 ml total.
- 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 slides in Fontana Silver Working Solution (Step #2) in a 45°C to 60°C water bath for 1 hour.
- Check slides microscopically; remove control, rinse in warm distilled water and confirm that 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 slides in Gold Chloride 0.2%, Aqueous for 10 minutes.
- Rinse well in distilled water.
- Counterstain in Sodium Thiosulfate 5%, Aqueous for 5 minutes.
- Rinse well in distilled water.
- Counterstain in Nuclear Fast Red Stain, Kernechtrot 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. Cleaning glassware with bleach is not equivalent to acid washing.
- Plastic (5500), plastic-tipped (5502, 5503), or paraffin coated metal forceps must be used with any silver solution to prevent precipitation of silver salts. No metals of any kind should be in contact with any silver solution. Only glass thermometers should be used.
- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

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