

Fontana Masson Stain Kit - Technical Memo

KIT INCLUDES:

	Part 9105A
Solution A: Silver Nitrate 10%, Aqueous	250 ml
Solution B: Ammonium Hydroxide 28-30%, ACS	250 ml
Solution C: Gold Chloride 0.2%, Aqueous	250 ml
Solution D: Sodium Thiosulfate 5%, Aqueous	250 ml
Solution E: Nuclear Fast Red Stain, Kernechtrot	250 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

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 bottle labels.

APPLICATION:

Newcomer Supply Fontana Masson Stain Kit procedure is used to demonstrate argentaffin substances such as melanin, argentaffin granules of carcinoid tumors, and some neurosecretory granules. This stain is not specific for melanin and argentaffin granules. 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 4 microns

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

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

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 Fontana Masson Ammoniacal Silver Working Solution in an acid cleaned Erlenmeyer flask:
 - a. Solution A: Silver Nitrate 10%, Aqueous; 25 ml
 - b. Add Solution B: Ammonium Hydroxide 28-30%, ACS drop by drop, mix with swirling motion until solution clouds, then clears. Do not add excess Ammonium Hydroxide.
 - c. Add more Solution A: Silver Nitrate 10%, Aqueous drop by drop until clear solution becomes slightly turbid or cloudy. The change is subtle.
 - d. Let solution stand for 2-4 hours before use.
 - e. For use; after standing, filter silver solution. Combine 20 ml of filtered silver solution with 40 ml of distilled water; 60 ml total.

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. Immerse slides in the Fontana Masson Ammoniacal Silver Working Solution (Step #3) in a 45°C-60°C water bath for 1 hour.

6. Check slides microscopically; remove control, rinse in warm distilled water. Confirm reaction is complete when granules are dark brown and background is colorless.
 - a. Return to heated Fontana Silver Working Solution for longer incubation if indicated.
7. Rinse well in three changes of distilled water.
8. Immerse in Solution C: Gold Chloride 0.2%, Aqueous; 10 minutes.
9. Rinse well in distilled water.
10. Place in Solution D: Sodium Thiosulfate 5%, Aqueous; 5 minutes.
11. Rinse well in distilled water.
12. Counterstain in Solution E: Nuclear Fast Red Stain, Kernechtrot for 5 minutes.
 - a. Shake solution well before use; do not filter.
13. Rinse well in distilled water.
 - a. See Procedure Note #5.
14. 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:

1. Acid clean all glassware/plasticware (12086) and rinse thoroughly in several changes of distilled water.
2. Plastic (5500), plastic-tipped 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.
3. Drain slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during procedure.
5. Wash well after Nuclear Fast Red Stain, Kernechtrot to avoid cloudiness in dehydration steps.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

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