

Melanin Control Slides – Technical Memo

CONTROL SLIDES: **Part 4430A** **Part 4430B**
10 Slide/Set 98 Slide/Set

Melanin Control Slides contain a section of positive staining skin.

PRODUCT DESCRIPTION:

The enclosed positive control slides are intended to be used to verify histological techniques and reagent reactivity. These slides are to be used for the qualitative purpose of determining positive or negative results, and are not intended to be used for any quantitative purpose. The first serial section within the control box is stained and provided for your reference. **Before using the unstained slides, review the enclosed stained slide with your pathologist to ensure that this tissue source is acceptable. Newcomer Supply will not accept a return with missing slides in the series. Newcomer Supply guarantees reactivity of these control slides for one year from the date of receipt. Revalidate after one year to verify continued reactivity. Store at 15-30°C in a light deprived and humidity controlled environment.**

These positive control slides were produced from human surgical or autopsy tissues under carefully controlled conditions. Reasonable measures are used to deliver quality control slides that are as consistent as possible. However, characteristics of quality control slides may be dissimilar due to variations in the reagents, stains, techniques, laboratory conditions, and tissue sources used. Newcomer Supply Laboratory uses a manual method of performing quality control procedures, specifically avoiding automation, in order to provide reactive control slides for even less aggressive methods of staining that our customers may be using.

CONTROL SLIDE VALIDATION:

With Fontana Masson Stain Kit:	Part 9105A	Individual Stain Solution
Solution A: Silver Nitrate 10%, Aqueous	250 ml	Part 13806
Solution B: Ammonium Hydroxide 28-30%, ACS	250 ml	Part 1006
Solution C: Gold Chloride 0.2%, Aqueous	250 ml	Part 11286
Solution D: Sodium Thiosulfate 5%, Aqueous	250 ml	Part 1389
Solution E: Nuclear Fast Red Stain, Kernechtrot	250 ml	Part 1255

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

APPLICATION:

Newcomer Supply Melanin Control Slides are for the positive histochemical staining of melanin in tissue sections.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns on Superfrost® Plus
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

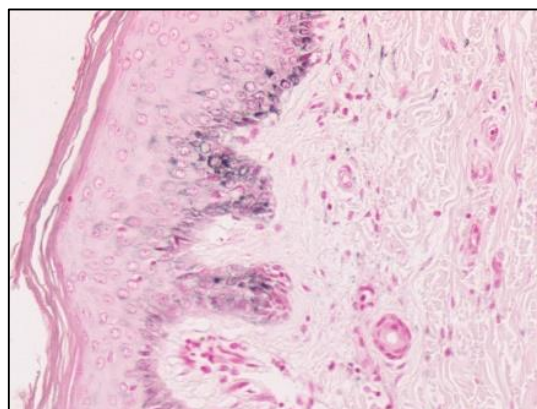
STAINING PROCEDURE:

- 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:
 - Solution A: Silver Nitrate 10%, Aqueous; 25 ml
 - Add Solution B: Ammonium Hydroxide 28-30%, ACS drop by drop, mixing with swirling motion until solution clouds, then clears. Use caution to not add too much Solution B: Ammonium Hydroxide 28-30%, ACS.
 - Add more Solution A: 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 the Fontana Silver Working Solution (Step #2) in a 45°C-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 Solution C: Gold Chloride 0.2%, Aqueous for 10 minutes.
- Rinse well in distilled water.
- Place slides in Solution D: Sodium Thiosulfate 5%, Aqueous for 5 minutes.
- Rinse well in distilled water.
- Counterstain in Solution E: 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	Black
Nuclei	Pink-red



PROCEDURE NOTES:

1. 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.
2. 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.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during staining 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. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 276-277.
2. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 286-287.
3. Modifications developed by Newcomer Supply Laboratory.