INTENDED USE

The Fontana-Masson Stain Kit is intended for use in the histological visualization of Argentaffin cells and Melanin in paraffin or frozen sections. Fontana-Masson Stain reagents are for "In Vitro Diagnostick Use." Fontana-Masson staining is used for the demonstration of argentaffin substances such as melanin, argentaffin granules of carcinoid tumors and some neurosecretory granules. Melanin typically stains in ~30 minutes while Argentaffin stains in ~60 minutes. Examine slides microscopically to ensure proper staining is achieved. Incubate longer if necessary to obtain optimal staining.

SPECIAL MATERIALS REQUIRED, BUT NOT PROVIDED:
Cut paraffin sections at 4 to 5 microns. Incorporate appropriate controls. Specimens should be considered potentially infectious.

STORAGE AND STABILITY:
Store unopened Fontana-Masson Stain kit and individual reagents in refrigerator (2-8°C). Reagents are stable until the expiration dates shown on the labels.

PREPARATION:
AMMONIACAL SILVER SOLUTION:
1. In a new or chemically cleaned glass Erlenmeyer flask, add 27 ml distilled water.
2. Pipette 9 ml Silver Nitrate Solution into the flask.
3. In a hood, while shaking or swirling the flask continuously, carefully add concentrated Ammonium Hydroxide, drop by drop, swirling gently after each drop addition.
4. Solution will turn dark brown and then gradually become transparent with a fine layer of sediment. Do not add excess Ammonium Hydroxide.
5. Solution is ready to use when all sediment dissolves. Use once and do not store. Other reagents are supplied ready for use.

PRECAUTIONS:
Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

Argentaffin and Melanin TISSUE-TROL™ control slides are paraffin embedded human tissue and should be considered potentially infectious.

PROCEDURE

SPECIMEN COLLECTION:
It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.

Fix specimen in 10% neutral buffered formalin, process and embed in paraffin. Cut paraffin sections at 4 to 5 microns. Incorporate appropriate controls.

SPECIAL MATERIALS REQUIRED, BUT NOT PROVIDED:
Positive Control Slides, such as Argentaffin TISSUE-TROL™ Catalog No. TTR013-25EA and Melanin TISSUE-TROL™ Catalog No. TTR014-25EA
Counterstain: Nuclear Fast Red Solution, Catalog No. N3020
Ammonium Hydroxide Solution, concentrated Formalin Solution, 10%
Reagent Alcohol
Xylene or xylene substitute
Coplin jars, chemically clean (plastic with vented lids for Microwave Procedure)
Microwave (for Microwave Procedure)

NOTE:
The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

PROCEDURE:
STD 1. Deparaffinize sections and hydrate to distilled water.
2. Place freshly prepared Ammoniacal Silver solution in a 58-60°C water bath and allow adequate time for the temperature to equilibrate.
3. Incubate slide in warmed Ammoniacal Silver solution for 0-60 minutes or until tissue section becomes yellow-brown in color. **Note:** Melanin typically stains in ~30 minutes while Argentaffin stains in ~60 minutes. Examine slides microscopically to ensure proper staining is achieved. Incubate longer if necessary to obtain optimal staining.
4. Rinse slides in several changes of distilled water.
5. Tone sections in Gold Chloride Solution for 30 seconds.
6. Rinse slides in several changes of distilled water.
7. Place slides in Sodium Thiosulfate Solution for 1-2 minutes.
8. Wash slides in tap water for 2 minutes.
9. Rinse slides in several changes of distilled water.
10. Counterstain with Nuclear Fast Red Solution for 5 minutes.
11. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.
12. Dehydrate in two changes each of 95% ethanol and absolute ethanol.
13. Clear in xylene and mount with synthetic resin.

MICROWAVE PROCEDURE:
1. Deparaffinize sections and hydrate to distilled water.
2. Place slides in 40 ml of Ammoniacal Silver solution contained in a plastic Coplin jar. Loosely cover Coplin jar with lid before placing in microwave oven, or use Coplin jars with holes drilled into the lids. Microwave on 400 watts for 1-2 minutes until tissue section becomes yellowish-brown in color.

PERFORMANCE CHARACTERISTICS

| Argentaffin Cells: Brown to Black |
| Melanin: Brown to Black |
| Background: Pink to Rose |

If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

TISSUE-TROL is a trademark of Sigma-Aldrich Co., LLC

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