Fontana-Masson Stain Kit
(For Argentaffin Cells and Melanin)

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
The Fontana-Masson Stain Kit is intended for use in the histological visualization of Argentaffin cells and Melanin in paraffin or frozen sections.

Argentaffin Cells: Black
Melanin: Black
Nuclei: Red
Cytoplasm: Light Pink

Control Tissue
Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981; Sheehan & Harpachak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 µm and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Recommended Positive Control
1. Hair Follicles
2. Skin for Melanin
3. Small Intestine for Argentaffin

Reagents Provided

<table>
<thead>
<tr>
<th>Kit Contents</th>
<th>Volume</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold Chloride Solution (0.2%)</td>
<td>125 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Silver Nitrate Solution (10%)</td>
<td>5 x 9 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Sodium Thiosulfate Solution (5%)</td>
<td>125 mL</td>
<td>RT</td>
</tr>
<tr>
<td>Nuclear Fast Red Solution</td>
<td>125 mL</td>
<td>RT</td>
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Storage and Handling
Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Prepare the Following Solutions Immediately Before Use
1. Prepare Ammoniacal Silver Solution immediately prior to use.
   i. In new or chemically cleaned glassware, mix 27 ml Distilled/Deionized water with one vial of Silver Nitrate Solution (10%) and blend completely.
   ii. Carefully add Concentrated Ammonium Hydroxide (Not included) one drop at a time, swirling gently after each drop.
   iii. Initially the mixture will turn dark brown and then gradually become transparent with a fine layer of sediment.
   iv. The solution is ready for immediate use when all sediment dissolves.

Staining Procedure
1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place freshly mixed Ammoniacal Silver Solution in a 58-60°C water bath and allow adequate time for temperature to equilibrate.
3. Incubate slide in warmed Ammoniacal Silver Solution for 30-60 minutes or until tissue section becomes yellowish/brown in color.
   **Note:** Melanin typically stains in 30 minutes while Argentaffin stains in 50-60 minutes
4. Rinse in several changes of distilled water.
5. Incubate slide in Gold Chloride Solution (0.2%) for 30 seconds.
6. Rinse in several changes of distilled water.
7. Incubate slide in Sodium Thiosulfate Solution (5%) for 1-2 minutes.
8. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.
9. Incubate slide in Nuclear Fast Red Solution for 5 minutes.
10. Rinse for 2 minutes in running tap water followed by 2 changes of distilled water.
11. Dehydrate very quickly in 3 changes of fresh Absolute Alcohol.

Limitations of the Procedure
1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

Precautions
1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.
7. Use in a chemical fume hood whenever possible.

Troubleshooting
If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

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References