Modified MOVATS Stain

Summary: The following table describes the staining color for various structures.

<table>
<thead>
<tr>
<th>Results</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei and Elastic Fibers</td>
<td>Black</td>
</tr>
<tr>
<td>Ground Substance and Mucin</td>
<td>Blue</td>
</tr>
<tr>
<td>Muscle</td>
<td>Red</td>
</tr>
<tr>
<td>Collagen and Reticular Fibers</td>
<td>Yellow</td>
</tr>
<tr>
<td>Fibrinioi and Fibrin</td>
<td>Intense Red</td>
</tr>
</tbody>
</table>

Protocol:

1) Deparaffinize and hydrate to distilled water
2) Preheat Alkaline Alcohol in 60 degree oven
3) Mordant in Bouins in microwave, covered, at Med power for 45 seconds. Let sit for 10 mins. (Do Not Dump)
4) Rinse in cold running water for 10 minutes
5) Sodium Thiosulfate for 5 minutes, wash for 5 minutes under cold running water
6) Stain in 1% Alcian Blue for 20 minutes (Do Not Dump)
7) Cold running water for 2-5 minutes
8) Place in preheated Alkaline Alcohol for 10 minutes. (in oven)
9) Cold running water for 2-5 minutes
10) Place in Movat’s Weigerts for 60 minutes (Do not Microwave)
11) Wash briefly in cold running water; rinse well in distilled water
12) Place in Crocein Scarlet/Acid Fuchsin for 1 min
13) Rinse in distilled water
14) Place in 5% Phosphotungstic Acid for 5 min
15) Transfer directly to 1% Acetic Acid for 5 minutes
16) Rinse well in distilled water
18) Dehydrate in 1 change of 95% ETOH, 2 changes of 100% ETOH 1 minute each
19) Alcoholic Saffron for 20 minutes (Do Not Dump)
20) Rinse in 2 changes of 100% ETOH for 1 minute each
21) Clear in histoclear (xylene) and mount

**Reagent Preparation:** *(This area may have several different preparations with the table of contents below.)*

**Alkaline Alcohol**
- 95% ETOH: 45 ml
- conc. Ammonium Hydroxide: 5 ml

**Bouins’s Solution**
- Picric Acid, saturated aqueous: 75 ml
- Formaldehyde 37-40%: 25 ml
- Glacial Acetic Acid: 5 ml

**1% Alcian Blue**
- Alcian Blue: 1 gm
- Distilled Water: 100 ml

**STOCK Weigert’s Hematoxylin**
- 2% Alcoholic Hematoxylin
  - Hematoxylin: 10 gm
  - 95% ETOH: 500 ml

**Ferric Chloride**
- Ferric Chloride Hexahydrate: 12.4 gm
- Distilled Water: 500 ml
- HCL, conc: 5 ml

**Iodine Solution**
- Iodine: 10 gm
- Potassium Iodide: 20 gm
- Distilled Water: 500 ml

*Dissolve the Potassium Iodide in a small amount of the distilled water. Add the iodine, when completely dissolved add the remainder of the distilled water.

**WORKING Weigert’s Solution**
- 2% Hematoxylin: 30 ml
- Ferric Chloride: 20 ml
- Iodine Solution: 10 ml

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*Mix in order, use immediately. Discard after use.

**STOCK** Crocein Scarlet 
- Crocein Scarlet MOO 3B: 1.0 gm 
- Distilled Water: 98.5 ml 
- Conc. Glacial Acetic Acid: 0.5 ml

**STOCK** Acid Fuchsin 
- Acid Fuchsin: 0.1 gm 
- Distilled Water: 99.5 ml 
- Conc. Glacial Acetic Acid: 0.5 ml

**WORKING** Crocein Scarlet/Acid Fuchsin 
- Stock Crocein Scarlet: 40 ml 
- Stock Acid Fuchsin: 10 ml

5% Phosphotungstic Acid 
- Phosphotungstic Acid: 5 gm 
- Distilled Water: 100 ml

1% Glacial Acetic Acid 
- Conc. Glacial Acetic Acid: 1 ml 
- Distilled water: 99 ml

Alcoholic Saffron 
- Saffron: 6 gm 
- Absolute Alcohol: 100 ml

* Store in airtight bottles in 55-60 degree oven for 48 hrs to extract saffron (Do not add additional alcohol to the solution as it evaporates because it will weaken the staining). Cool the extract to room temperature, filter through a coarse filter paper in a covered funnel and store in the filtrate in a dark tightly stoppered container.

5% Sodium Thiosulfate 
- Sodium Thiosulfate: 5 gm 
- Distilled Water: 100 ml

0.1% Sirius Red Solution 
- Sirius Red Dye: 0.1 gm 
- Saturated Picric Acid: 100 ml

*Store in refrigerator, stable for 4-6 months.*