**F-367**  
**MASSON’S TRICHOROME FOR CONNECTIVE TISSUE**

**Fixation:**  
Bouin’s Fixative (F-40) or **10% Buffered Formalin** (F-113)

**Sections:**  
Paraffin cut at 6 micrometers.

**PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Mordant in Bouin’s Fixative (F-367-1) for 1 hour at 56°C or overnight at room temperature.
3. Cool and wash in running water until yellow color disappears.
4. Rinse in distilled water.
5. Place in Weigert’s Iron Hematoxylin Solution for 10 minutes.

**To prepare Weigert’s Iron Hematoxylin Working Solution:**

Mix equal parts of **Weigert’s A** (F-367-2A)  
with equal parts of **Weigert’s B** (F-367-2B)

6. Wash in running tap water for 10 minutes.
7. Rinse in distilled water.
8. Place in **Biebrich Scarlet-Acid Fuchsin** (F-367-3) for 15 minutes.
9. Rinse in distilled water.
10. Place in **Phosphotungstic-Phosphomolybdic Acid** (F-367-4) for 10 to 15 minutes.
11. Place in **Aniline Blue Solution** (F-367-5) for 10-20 minutes.
12. Rinse in distilled water.
13. Place in **Acetic Acid, 1%** (F-367-6) for 3 to 5 minutes. Discard solution.
14. Dehydrate in 95% Alcohol, Absolute Alcohol, and clear in Xylene, 2 changes each.
15. Mount.

Shorten staining times when using 2-4 micron sections of renal biopsy specimens:

Biebrich Scarlet-Acid Fuchsin..................5 minutes  
Phosphomolybdic Acid-Phosphotungstic Acid......10-12 minutes  
Aniline Blue Solution...............................3 minutes

**Stain Results:**

<table>
<thead>
<tr>
<th>Stain Location</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclei</td>
<td>Black</td>
</tr>
<tr>
<td>Cytoplasm, Keratin, Muscle Fibers</td>
<td>Red</td>
</tr>
<tr>
<td>Collagen, Mucin</td>
<td>Blue</td>
</tr>
</tbody>
</table>

**Note:** To insure proper mordanting. Bouins Fixative should be preheated to 58-60°C.

**References:**