# ROWLEY BIOCHEMICAL INC.

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## F-362 MASSON'S TRICHROME STAIN

**<u>Fixation</u>**: **<u>Bouin's</u>** (F-40) for three days, **<u>Moller</u>** (F-190) for 24 hours, or 10% **<u>Neutral Formalin</u>** (F-113).

**Sections:** Paraffin sections@ 6 microns

#### **PROCEDURE:**

1. Deparaffinize by xylene, alcohol and distilled water.

2. Mordant 5 minutes in **Ferric Ammonium Sulfate, 5%** (F-362-9), previously heated to 45°C or, if Formalin fixed, in **Bouin's Solution,** (F-362-9a) for one hour @ 56°C.

2A. If using a microwave-Mordant in **Bouin's Solution** (F-362-9A), for 35 seconds in the microwave oven. Let the solution stand for 2 minutes at room temperature.

- 3. Wash in running water.
- 4. Stain for 5 minutes in **Regauds' Hematoxylin** (F-362-1), and rinse in 95% alcohol or stain in Weigert's working solution for 5-10 minutes, wash in running water 10 minutes and rinse in distilled water.

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  - 4A. If using a microwave, stain in working Weigert's solution for 15 seconds in the microwave oven, wash in running water 10 minutes and rinse in distilled water.
- 5. Differentiate in <u>Picric Alcohol</u> (F-362-2). Wash in running tap water. (This step may be omitted if Weigert's Iron Hematoxylin is used in step four).
- 6. Stain 5 minutes in **Ponceau Acid Fuchsin** (F-362-3). Rinse in distilled water.
- 7. Differentiate five minutes in **Phosphomolybdic Acid**, 1% aq (F-362-7).
- 8. Pour on Aniline Blue Solution (F-362-4), without rinsing. Allow to stand for 5 minutes. Rinse in distilled water.

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  - 8A. If using a microwave oven-pour on <u>Aniline Blue Solution</u> (F-362-4), without rinsing. Microwave for 15 seconds. Rinse in distilled water.
- 9. Return to **Phosphomolybdic Acid**, 1% (F-362-7), for 5 minutes. Place in **Acetic Acid**, 1% (F-362-8), for 5 minutes.
- 10. Dehydrate in 95% alcohol, absolute alcohol, clear in xylene. Mount with **Permount** (M-18)

### **Stain Results:**

Nuclei	Black
Argentaffin Granules	Black or Red
Cytoplasm, Keratin, Intercellular Fibers and	Red
Neuroglial Fibers	
Collagen	Blue

#### References:

Masson, P.J., <u>J. Techn. Methods</u>: 12:75 (1929)

Clark, G.: <u>Staining Procedures</u>: 3<sup>rd</sup> ed., Williams & Wilkins Co. Baltimore,

p. 57c. 1973

Ed.G.Luna AFIP Manual of Histological Staining Methods, 3<sup>rd</sup> ed., McGraw-Hill Publications, New York, p. 94 c. 1968