ROWLEY BIOCHEMICAL INC.

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F-367 MASSON'S TRICHROME FOR CONNECTIVE TISSUE

<u>Fixation</u>: **<u>Bouin's Fixative</u>** (F-40) or **<u>10% Buffered Formalin</u>** (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 <u>Weigert's A</u> (F-367-2A) with equal parts of <u>Weigert's B</u> (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:

BiebrichScarlet- Acid Fuchsin	.5 minutes
Phosphomolybdic Acid-Phosphotungstic Acid10-12	2 minutes
Aniline Blue Solution	3 minutes

Stain Results:

Nuclei	Black
Cytoplasm, Keratin, Muscle Fibers	Red
Collagen, Mucin	Blue

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

References:

Sheehan and Hrapchak, Theory and Practice of Histotechnology, St. Louis, The Mosby Company 1980, p.190.