MASSON TRICHROME STAINING

(Michelle's protocol, 9/01)

REAGENTS NEEDED:

- Sigma Accustain Trichrome Stain Kit (Catalog #HT15) contains:
 - **Biebrich Scarlet-Acid Fuchsin Solution** (# HT15-1, 0.9% biebrich scarlet, 0.1% acid fuchsin, 1% acetic acid), **Phosphotungstic Acid Solution** (#HT15-2, 10% phosphotungstic acid), **Phosphomolybdic Acid Solution** (#HT15-3, 10% phosphomolybdic acid), and **Aniline Blue Solution** (#HT15-4, 2.4% aniline blue, 2% acetic acid)
- Bouin's Solution (Sigma Catalog #HT10132-1L or HT101128-4L)
- Weigert's Iron Hematoxylin Set (Sigma catalog #HT10-79)
- 1. Deparaffinize slides and rehydrate sections:
 - 3 x 3' Xylene (blot excess xylene before going into ethanol)
 - 3 x 3' 100% ethanol
 - 1 x 3' 95% ethanol
 - 1 x 3' 80% ethanol
 - 1 x 5' deionized H₂O
- Mordant in Bouin's Solution at room temperature overnight in a hood. Be careful, Bouin's solution is hazardous and the picric acid, when in less than 10% water, is very explosive. Used Bouin's solution should be placed in an appropriate waste container.
 - ** Bouin's Solution intensifies the final coloration of the tissue.

3. Wash slides in running tap water to remove yellow color from sections. Rinse briefly in distilled water.

4. Stain in Working Weigert's Iron Hematoxylin Solution for 5 minutes. Make Hematoxylin Solution fresh by adding equal volumes of Solution A (1% Hematoxylin in 95% EtOH) and Solution B (1.2% Ferric Chloride and 1% Acetic Acid in distilled water). The working solution is good for approximately 10 days.

- ** Hematoxylin stains nuclei blue-black.
- 5. Wash in running tap water for 5 minutes. Rinse in deionized water.
- 6. Stain in Biebrich Scarlet-Acid Fuchsin for 5 minutes.
 - Decreased red staining usually indicates that the staining solution has aged or been overused and should be discarded.
 - ** Beibrich scarlet-acid fuchsin stains cytoplasm and muscle red.
- 7. Rinse in deionized/distilled water.

8. Place the slides in Phosphomolybdic/Phosphotungstic Acid Solution for 5-10 minutes. Freshly prepare Working Phosphotungstic/Phosphomolybdic Acid Solution by mixing 1 volume of Phosphotungstic Acid Solution and 1 volume of Phosphomolybdic Acid Solution with 2 volumes of distilled water. Discard after one use. Formation of a precipitate in Phosphomolybdic Acid Solution does not affect performance.

** This allows for uptake of the aniline blue stain.

- Stain sections in Aniline Blue Solution for 5 minutes.
 **Aniline blue stains collagen blue.
- 10. Rinse slides briefly in distilled water.
- 11. Place slides in 1% acetic acid solution for 3-5 minutes. Discard this solution.
 ** Rinsing in acetic acid after staining renders the shades of color more delicate and transparent.

** If blue staining of connective tissue appears faded, the section has probably been overdifferentiated in the acetic acid solution.

12. Dehydrate to xylene.

2 x 3′	95% ethanol
2 x 3′	100% ethanol (blot excess ethanol before going into xylene)
3 x 5′	Xylene

- 13. Leave slides in xylene overnight to get good clearing of the ethanol.
- 14. Coverslip slides using Permount or Polymount (xylene based).
 - •Place a drop of Permount on the slide using a glass rod.
 - •Angle the coverslip and let fall gently onto the slide.

•Allow the Permount to spread beneath the coverslip, covering all the tissue. •Dry overnight in the hood.

Representative Images:

