

MASSON'S TRICHOME METHOD FOR CONNECTIVE TISSUE

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of collagen and smooth muscle in proximity.
PRINCIPLE:	Post fixation in Bouin's increases the intensity of the stain. The highly negative nuclei are then stained by a positive iron hematein lake. An acid dye is applied to the section which binds all acidophilic elements including both collagen and muscle. The Phosphotungstic Phosphomolybdic Acid then displaces the acid dye from the collagen. Collagen is more permeable than cytoplasm and thus the large phosphotungstic phosphomolybdic molecule cannot displace the acid dye from the cytoplasm. It also acts as a mordant for the Aniline Blue to bind to collagen. Acetic Acid clears the slide of any loosely bound dye.
CONTROL:	Uterus, appendix <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs025.</i>
SPECIMEN PREPARATION:	Formalin or Bouin's fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	1. Bouin's Fixative Item# s129 2. Weigert's Iron Hematoxylin Solution Set (A & B) Item# s216B <i>Working Solution:</i> Mix equal parts of solutions A & B before use. 3. Biebrich Scarlet Acid Fuchsin Solution Item# s125 4. Phosphotungstic Phosphomolybdic Acid Item# s255 5. Aniline Blue Masson's Trichrome Item# s116 6. Acetic Acid 1% Aqueous Item# s100 <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	To insure proper mordanting, Bouin's Fixative should be preheated to 58-60°C.
REFERENCE:	Bancroft, J. D. & Stevens, A. <u>Theory and Practice of Histological Techniques</u> . 4th Ed. Churchill Livingstone. New York. 1996. pp. 102-104.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. Mordant in Bouin's Fixative for 1 hour at 56°C under a hood.
3. Cool and wash in running water until most is washed out.
4. Rinse in distilled water.
5. Place in Weigert's Iron Hematoxylin Working Solution for 5 minutes.
6. Wash in running water for 5 minutes.
7. Rinse in distilled water.
8. Place in Biebrich Scarlet Acid Fuchsin for 2 minutes. Save solution.
9. Rinse in distilled water.
10. Place in Phosphotungstic Phosphomolybdic Acid for 10-15 minutes.
11. Place in Aniline Blue Solution for five minutes (for central nervous system, leave for 15-20 minutes). Save solution.
12. Rinse in distilled water.
13. Place in Acetic Acid 1% Aqueous for 3-5 minutes. Discard solution.
14. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
15. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Nuclei.....Black
 Cytoplasm, Keratin Muscle Fibers and Intercellular Fibers.....Red
 Collagen.....Blue

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