

MASSON'S TRICHROME METHOD FOR CONNECTIVE TISSUE (Microwave)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of collagen and smooth muscle 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 Acid then displaces the acid dye from the collagen. Collagen is more permeable than cytoplasm and thus the large phosphotungstic 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:	Bouin's Fixative 10% Buffered Formalin 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 for use. 3. Biebrich Scarlet Acid Fuchsin Solution Item# s125 4. Phosphotungstic Acid 5% Aqueous Item# s2090 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:	<i>Variations in timing may occur due to the power wattage of the microwave oven. Provided times and power levels are based on 1000 watt microwave oven.</i>
REFERENCE:	Clark, George. <i>Staining Procedures</i> . 4th Ed. Williams & Wilkins. Baltimore, MD. 1981. pp. 119-120.

STAINING PROCEDURE:

1. Deparaffinize and hydrate slides to distilled water.
2. Place 50 mL of Bouin's Fixative in plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave for an additional 20 seconds. Stir again and add slides to hot solution for 3-5 minutes.
3. Rinse in running water until all yellow disappears from sections.
4. Place 50 mL of Weigert's Iron Hematoxylin Working Solution in plastic coplin jar (loosely apply cap) and microwave for 25 seconds. Stir and re-microwave for 15 seconds. Stir again and add slides to hot solution for 4-5 minutes.
5. Rinse well in running water. Check control. If additional staining time is needed place slides back in hot solution until desired intensity is reached.
6. Place 50 mL Biebrich Scarlet Acid Fuchsin Solution in plastic coplin jar (loosely apply cap) and microwave for 30 seconds. Place slides in hot solution for 30-60 seconds.
7. Place 50 mL of Phosphotungstic Acid 5% Aqueous in plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave for 15 seconds. Stir again and add slides to hot solution for 5 minutes.
8. Rinse well in distilled water.
9. Counterstain in Aniline Blue Masson's Trichrome by dipping slides one at a time until desired intensity is reached (approximately 5-10 dips).
10. Place 50 mL of Acetic Acid 1% Aqueous in plastic coplin jar and microwave for 30-45 seconds. Stir and add slides to hot solution for 1-2 minutes.
11. Dehydrate, clear and mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

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

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