

CHROMOTROPE 2R WITH ANILINE BLUE TRICHROME STAIN

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of collagen and smooth muscle proximity.
PRINCIPLE:	Post fixation in Picric Acid Saturated increases the intensity of the stain. The positive nuclei are then stained by an anion iron hematein lake. An acid dye is applied to the section which binds all acidophilic elements including both collagen and muscle. 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:	Formalin fixed, paraffin embedded sections cut at 6 micrometers
SOLUTIONS:	1. Picric Acid Saturated Aqueous Item# s257 2. Gomori's One Step Trichrome with Aniline Blue Item# s1816 3. Weigert's Iron Hematoxylin Solution Set (A&B) Item# s216B <u>Working Solution:</u> Mix equal parts of A and B for use. 4. Acetic Acid 0.5% Aqueous Item# s2015 <i>Solutions can be purchased separately from Poly Scientific.</i>
NOTES:	
REFERENCE:	Luna, Lee G. <u>Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology</u> . 3rd Ed. McGraw-Hill Book Co. New York. 1968. p. 98.

STAINING PROCEDURE:

1. Deparaffinize and hydrate to water.
2. Saturate in Picric Acid at 60°C for 15 minutes.
3. Wash well in running water until most of the Picric Acid is washed out.
4. Weigert's Iron Hematoxylin Working Solution for 10 minutes.
5. Wash well in running water.
6. Trichrome Stain for 15 minutes.
7. Place in Acetic Acid 0.5% Aqueous for 2 minutes. If sections are too dark, differentiate in Acetic Acid 1% Aqueous to which 0.7 grams of Phosphotungstic Acid has been added. Rinse in distilled water.
8. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
9. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Muscle Fibers Red
 Collagen..... Blue
 Nuclei..... Blue to Black

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