

BENNHOLD'S MODIFIED METHOD FOR AMYLOID (CONGO RED) (Microwave)

PURPOSE:	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of amyloid in tissue.
PRINCIPLE:	Congo Red has a highly selective affinity for amyloid. This is due to the beta-pleated arrangement of the amyloid and the planar shape of the Congo Red molecule. The Congo Red inserts itself in-between the pleats of the amyloid and is held by hydrogen bonding. The wash with Alkaline Alcohol removes excess Congo Red from the section.
CONTROL:	Tissue with a known presence of amyloid <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs001.</i>
SPECIMEN PREPARATION:	Formalin fixed, paraffin embedded sections cut at 8-10 micrometers
SOLUTIONS:	1. Congo Red 1% Aqueous Item# s167 2. Bluing Solution 1% Lithium Carbonate Item# s127 3. Mayer's Modified Hematoxylin Item# s216 4. Acid Alcohol 1% Item# s104 <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:	Bancroft, J. D. & Stevens, A. <u>Theory and Practice of Histological Techniques</u> . 4th Ed. Churchill Livingstone. New York. 1996. pp 311-312.

STAINING PROCEDURE:

1. Deparaffinize and hydrate slides to distilled water.
2. Place 50 mL of Congo Red 1% Aqueous in a plastic coplin jar (loosely apply cap) and microwave for 25 seconds. Stir and re-microwave an additional 25 seconds. Stir and place slides in hot solution for 10 minutes.
3. Differentiate in Bluing Solution 1% Lithium Carbonate until stain does not run and rinse in distilled water.
4. Place 50 mL of Mayer's Modified Hematoxylin in a plastic coplin jar (loosely apply cap) and microwave for 20 seconds. Stir and re-microwave an additional 10 seconds. Stir and place slides in solution for 1 minute and rinse in water.
5. Differentiate in Acid Alcohol 1% and rinse in water.
6. Blue in Bluing Solution 1% Lithium Carbonate and rinse in water.
7. Dehydrate in 95% Alcohol, Absolute Alcohol and clear in Xylene, 2 changes each.
8. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

RESULTS:

Amyloid..... Orange Red to Red
Nuclei..... Blue

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