

**ALKALINE CONGO RED METHOD FOR AMYLOID**

<b>PURPOSE:</b>	For In Vitro Diagnostic Use: Intended for the qualitative demonstration of amyloid in tissue.
<b>PRINCIPLE:</b>	Amyloid is selectively stained by Congo Red due to the linear configuration of the dye molecule. This permits hydrogen bonding of the azo and amine groups of the dye to the similarly spaced hydroxyl radical of the amyloid. Pretreatment in alkaline alcohol releases native internal hydrogen bonding and creates, therefore, more sites for potential binding with the dye.
<b>CONTROL:</b>	Any tissue known to contain amyloid <i>Control Slides can be purchased from Histology Control Systems. See inside back cover, Item# cs001.</i>
<b>SPECIMEN PREPARATION:</b>	Alcohol or Carnoy's Fluid preferred fixed, paraffin embedded sections cut at 6-10 micrometers
<b>SOLUTIONS:</b>	1. Harris Hematoxylin with Glacial Acetic Acid Item# s212A 2. Sodium Chloride Saturated in 80% Reagent Alcohol Item# s2498 3. Congo Red Stock Solution Item# s2247 4. Sodium Hydroxide 1% Aqueous Item# s1918A  <i>Solutions can be purchased separately from Poly Scientific.</i>
<b>NOTES:</b>	Following Congo Red Staining, bright apple-green birefringence exhibited under polarized light is considered specific for amyloid.
<b>REFERENCE:</b>	Carson, F. <u>Histotechnology: A Self-Instructional Text</u> . ASCP Press. Chicago, IL. 1990. pp. 132-134.

**STAINING PROCEDURE:**

1. Deparaffinize and hydrate to distilled water.
2. Place in Harris Hematoxylin with Glacial Acetic Acid for 2 1/2 minutes.
3. Wash in running water for several minutes.
4. Place slides in freshly made Alkaline Salt Solution for 20 minutes.  
Alkaline Salt Solution: Filter and use within 15 minutes.  
Sodium Chloride Saturated in 80% Reagent Alcohol ..... 50 mL  
Sodium Hydroxide 1% Aqueous..... 0.5 mL
5. Place slides in freshly made Working Congo Red Solution for 20 minutes.  
Working Congo Red Solution: Filter and use within 15 minutes.  
Congo Red Stock Solution.....50 mL  
Sodium Hydroxide 1% Aqueous.....0.5 mL
6. Dehydrate rapidly in 3 changes of Absolute Alcohol, 5-6 good dips in each.
7. Clear in 2-3 changes of Xylene.
8. Mount with Poly Mount (Item# s2153) or any other acceptable mounting medium.

**RESULTS:**

Amyloid ..... Deep Pink to Red  
Elastic Tissue ..... Pale Pink  
Nuclei ..... Blue

*Poly Scientific R&D Corp.*

Revision: B-18

