

Amyloid, Bennhold Congo Red Stain Kit - Technical Memo

KIT INCLUDES:

	Part 9103A
Solution A: Congo Red Stain 1%, Aqueous	250 ml
Solution B: Alkaline Alcohol	250 ml
Solution C: Hematoxylin Stain, Mayer Modified	250 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Coplin Jar, Plastic	Part 5184 (for microwave modification)

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Amyloid, Bennhold Congo Red Stain Kit procedure, with included microwave modification, is used for identifying the extraneous protein deposits in amyloidosis. This procedure can identify minute amounts of amyloid. The use of polarizing lenses is the essential technique for visualizing amyloid positive areas and/or to confirm negativity.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)

Technique: Paraffin sections cut at 8-10 microns

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

STAINING PROCEDURE:

1. Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. See Procedure Notes #1 and #2.
2. Place slides in Solution A: Congo Red Stain 1%, Aqueous for 1 hour.

Microwave Modification: See Procedure Note #3.

 - a. Place slides in a plastic Coplin jar containing Solution A: Congo Red Stain 1%, Aqueous and microwave at 70°C for 3 minutes.
3. Rinse in two to three changes of tap water; rinse in distilled water.
4. Differentiate in Solution B: Alkaline Alcohol, 5 to 30 seconds, agitating constantly until slide background is cleared of Solution A: Congo Red Stain 1%, Aqueous.
5. Rinse in two to three changes of tap water; rinse in distilled water.
6. Counterstain with Solution C: Hematoxylin Stain, Mayer Modified, 3 to 5 minutes, depending on preference of nuclear stain intensity.
7. Wash in running tap water for 5 to 10 minutes.
8. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Light Field Microscopy:	
Amyloid	Pink to red
Nuclei	Blue
Polarized Light:	
Amyloid fluorescence	Apple green

PROCEDURE NOTES:

1. Drain staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
4. For optimal results sections should be cut at 8-10 microns. This will provide more intense staining and allow smaller amyloid deposits to be identified. Sections cut too thin may show faint staining and sections cut thicker than 8-10 microns may display yellow birefringence.
5. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 366-367.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 177-178.
3. Modifications developed by Newcomer Supply Laboratory.