

Amyloid, Puchtler Congo Red Stain Kit - Technical Memo

KIT INCLUDES:

Solution A: Hematoxylin Stain, Harris Modified
Solution B: Sodium Hydroxide 1%, Aqueous
Solution C: Congo Red Stain, Alcoholic

Part 9104A

250 ml
25 ml
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

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

APPLICATION:

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

METHOD:

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

Technique: Paraffin sections cut at 8-10 microns

a. See Procedure Note #1.

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. Prepare fresh Congo Red Working Stain Solution; combine and mix well.
 - a. Solution C: Congo Red Stain, Alcoholic 40 ml
 - b. Solution B: Sodium Hydroxide 1%, Aqueous 0.4 ml
 - c. See Procedure Note #2.
2. 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 #3 and #4.
3. Stain sections in Solution A: Hematoxylin Stain, Harris Modified for 30 seconds to 1 minute, depending on preference of nuclear stain intensity.
4. Wash in running tap water for 1 minute; rinse in distilled water.
 - a. Do not differentiate or use a bluing agent after hematoxylin staining.
5. Place slides in 95% ethyl alcohol; 1-2 dips.
6. Stain slides in fresh Congo Red Working Stain Solution (Step #1) for 20-30 minutes.
 - a. See Procedure Note #5.
7. Dehydrate quickly in two changes each of 95% and 100% ethyl alcohol; 10 dips each. 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. 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 that are too thin may show faint staining and sections that are thicker than 8-10 microns may display yellow birefringence.
2. Solution C: Congo Red Stain, Alcoholic is a saturated solution and dye may precipitate out over time. If excess precipitate occurs, filter the Congo Red Working Stain Solution prior to use.
3. Drain staining rack/slides after each step to prevent solution carry over.
4. Do not allow sections to dry out at any point during staining procedure.
5. To increase staining intensity, exposure in Congo Red Working Stain Solution can be extended up to 50 minutes.
6. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 270-272.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 154-155.
3. Churukian, Charles. "Improved Puchtler's Congo Red Method for Demonstrating Amyloid." *The Journal of Histotechnology* 23.2 (2000): 139-141.
4. Sheehan, Deza C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 177-178.
5. Modifications developed by Newcomer Supply Laboratory.