

## 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 are two complimentary unstained positive control slides 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](http://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 an 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.

**PRESTAINING PREPARATION:**

1. If necessary, heat dry tissue sections/slides in oven.
2. 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 #1.*

**STAINING PROCEDURE:**

3. 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 #2 and #3.*
4. Stain in Solution A: Hematoxylin Stain, Harris Modified for 30 seconds to 1 minute.
5. Wash in running tap water for 1 minute; rinse in distilled water.
  - a. *Do not differentiate or blue after hematoxylin staining.*
6. Place in 95% ethyl alcohol; 1-2 dips.
7. Stain in fresh Congo Red Working Stain Solution (Step #2) for 20-30 minutes.
  - a. *See Procedure Note #4.*
8. 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
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**PROCEDURE NOTES:**

1. Solution C: Congo Red Stain, Alcoholic is a saturated solution and dye may precipitate. Excess precipitate can be filtered out.
2. Drain slides after each step to prevent solution carry over.
3. Do not allow sections to dry out at any point during procedure.
4. Exposure in Congo Red Working Stain Solution can be extended up to 50 minutes to increase staining intensity.
5. For optimal results sections should be cut at 8-10 microns to provide more intense staining and allow smaller amyloid deposits to be identified. Sections cut too thin may show faint staining and sections that are thicker than 8-10 microns may display yellow birefringence.
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, Dezna 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.