

Congo Red Stain Set, Puchtler, Amyloid - Technical Memo

SET INCLUDES:

	Part 1037A	Part 1037B
Solution A: Sodium Hydroxide 1%, Aqueous	25 ml	50 ml
Solution B: Congo Red Stain, Alcoholic	250 ml	500 ml

Additionally Needed:

Amyloid, Animal Control Slides	Part 4031	or	Amyloid Control Slides	Part 4030
Xylene, ACS	Part 1445			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Alcohol, Ethyl Denatured, 95%	Part 10842			
Hematoxylin Stain, Harris Modified	Part 1201			

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

APPLICATION:

Newcomer Supply Congo Red Stain Set, Puchtler, Amyloid is used in identifying extraneous protein deposits in amyloidosis. The use of polarizing lenses is essential for visualizing amyloid positive areas or to confirm negativity.

METHOD:

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

Technique: Paraffin sections cut at 8 microns

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

All Newcomer Supply Stain Sets are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure. Some solutions in the set 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 B: Congo Red Stain, Alcoholic 40 ml
 - b. Solution A: 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 Hematoxylin Stain, Harris Modified (1201) for 30 seconds to 1 minute.
5. Wash in running tap water for 1 minute; rinse in distilled water.
 - a. Do not differentiate or use a bluing agent.
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

PROCEDURE NOTES:

1. Solution B: Congo Red Stain Alcoholic is a saturated solution and dye may precipitate, which 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 microns to provide more intense staining and allow smaller amyloid deposits to be identified.
6. If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

REFERENCES:

1. Carson, Freida L. and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 154-155.
2. Churukian, Charles. "Improved Puchtler's Congo Red Method for Demonstrating Amyloid." *The Journal of Histotechnology* 23.2 (2000): 139-141.
3. Sheehan, Dezna C. and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 177-178.
4. Modifications developed by Newcomer Supply Laboratory.