2505 Parview Road • Middleton, WI 53562-2579 • 800-383-7799 • www.newcomersupply.com • info@newcomersupply.com

Part 1037
Revised December 2019

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
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 for identifying extraneous protein deposits in amyloidosis, as well as minutes amount of amyloid. 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 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.

STAINING PROCEDURE:

1. If necessary, heat dry tissue sections/slides in oven.
2. Prepare fresh Congo Red Working Stain Solution; 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.*
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. *Extend up to 50 minutes for more intense stain results*.
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. If excess precipitate forms in Solution B: Congo Red Stain, filter the Congo Red Working Stain Solution prior to use.
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. 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.
5. If using a xylene substitute, closely follow the manufacturer's recommendations 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.