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Part 1037
Revised December 2016

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 bottle labels.

APPLICATION:

Newcomer Supply Congo Red Stain Set, Puchtler, Amyloid is used for 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 Sets are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the set may contain extra volumes.

STAINING PROCEDURE:

1. 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 #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 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 B: 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, 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.