

Thermo Scientific Richard-Allan Scientific Chromaview – Advanced Testing Amyloid Stain – Congo Red Instructions for Use

Technical Discussion

Microtomy

Cut sections at 7-10 microns.

Fixation

No special requirements; formalin fixation is adequate.

Quality Control

A section containing amyloid should be used.

Technical Procedure

Working Solution (Alkaline Congo Red)

Congo Red Solution..... 50ml
Sodium Hydroxide Solution.....10 drops
Mix well

Standard Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Stain sections in working Alkaline Congo Red Solution for 20 minutes.
3. Rinse sections in deionized water for 1 minute.
4. Counterstain sections in Modified Mayer's Hematoxylin for 10 minutes.
5. Rinse sections in deionized water for 1 minute.
6. Place sections in Bluing Reagent for 1 minute.
7. Rinse sections in deionized water for 1 minute.
8. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
9. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Microwave Staining Protocol

1. Deparaffinize and hydrate sections to deionized water.
2. Place 50ml of working Alkaline Congo Red Solution in a plastic coplin jar with lid applied loosely.
3. Microwave jar at 60% power for 30 seconds or as needed for a final temperature of 70°C.
4. Remove jar from oven and add slides to jar. Let stand for 5 minutes.
5. Rinse sections in deionized water for 1 minute.
6. Counterstain sections in Modified Mayer's Hematoxylin for 10 minutes.
7. Rinse sections in deionized water for 1 minute.
8. Place sections in Bluing Reagent for 1 minute.
9. Rinse sections in deionized water for 1 minute.
10. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
11. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results

Amyloid – Red to Pink-Red

Nuclei – Blue

Elastic Fibers – Light Red

Amyloid – Polarized light - Apple Green

Discussion

All staining reagents should be stored at room temperature. The Amyloid staining reagents are for "In Vitro" use only. Refer to the Material Safety Data Sheet for Health and Safety Information. All reagents are stable and should not form precipitants under ordinary storage parameters. The Working Alkaline Congo Red Solution should be discarded after use. The Modified Mayer's Hematoxylin Solution can be filtered and reused. These stains should not be diluted and are ready for use. All dyes used in these formulations are certified by the Biological Stain Commission.

Technical Comments

More intense amyloid staining is achieved with the room temperature procedure. When examining the stained slides under a polarizing microscope, the amyloid shows a green birefringence. The microwave protocol was developed using a 1200 watt microwave oven. Microwave frequencies vary from model to model. It may be necessary to adjust power levels or times to achieve desired results.

Probable Mode of Action

Under certain pathologies, amyloid, a fiber-like protein, can be deposited within tissues. Detection of amyloid by the use of green birefringence is considered the most specific technique (Carson). Hydrogen bonds between adjacent amyloid fibers are broken by the use of the sodium hydroxide. This allows for more binding sites for the Congo Red dye.

References

1. Bancroft, J.D. and Stevens, A. Theory and Practice of Histological Techniques. Churchill Livingstone, New York, NY, 1977.
2. Sheehan, D.C. and Hrapchak, B.B. Theory and Practice of Histotechnology, 2nd Edition. Mosby, St. Louis, MO, 1980.
3. Thompson, C.C. Selected Histochemical and Histopathological Methods. Springfield, IL, 1966.
4. Ramakrishnan, T., et. al.: Bacteriol. Rev. 36: 1976.
5. Carson. F.L. Histotechnology: A Self-Instructional Text, 2nd Edition. ASCP Press. Chicago, 1997.

Order Information

Product	Size	Qty.	REF
Amyloid Stain Kit	1 Kit	1	87003
Sodium Hydroxide Solution	8ml	1	88004
Congo Red Solution	500ml	1	88005
Modified Mayer's Hematoxylin	500ml	1	88006

