

Thermo Scientific Richard-Allan Scientific Chromaview – Advanced Testing Reticulin Silver Stain Instructions for Use

Technical Discussion

Microtomy

Cut sections at 4-6 microns.

Fixation

No special requirements; 10% Neutral Buffered Formalin is preferred.

Quality Control

A section of liver should be used.

Working Ammoniacal Silver Solution

Prepare just before use:

1. Place 5ml of Silver Nitrate 10% in a small clean flask.
2. Add Ammonium Hydroxide drop by drop while stirring until the precipitate that forms just barely dissolves. (Be careful to not over-titrate).
3. Add 5ml of 3% Sodium Hydroxide.
4. Add Ammonium Hydroxide drop by drop until the precipitate is completely dissolved.
5. Add Silver Nitrate 10% drop by drop until 1 drop causes the solution to become permanently cloudy. (A faint cloudiness is desired).
6. Dilute the solution to 50ml with deionized water and transfer to a clean coplin jar.
Note: Discard the Ammoniacal Silver Solution after use. Do not reuse.

Technical Procedure

1. Deparaffinize sections and hydrate to deionized water.
2. Oxidize sections in 1% Potassium Permanganate Solution for 5 minutes.
3. Rinse in deionized water for 1 minute.
4. Bleach in 1% Oxalic Acid for 2 minutes, or until sections are colorless.
5. Rinse in deionized water for 1 minute.
6. Sensitize sections in 2.5% Ferric Ammonium Sulfate for 15 minutes.
7. Rinse in several changes of deionized water.
8. Impregnate sections in Working Ammoniacal Silver Solution for 2 minutes.
9. Rinse well with deionized water.
10. Dip slide quickly in Reducing Solution until solution sheets and section has turned uniformly dark brownish-black. Allow slides to remain in the solution for an additional 1-2 minutes.
11. Rinse in deionized water for 1-2 minutes.
12. Tone in 0.1% Gold Chloride for 5-10 minutes. Background should appear gray-lavender with black reticular fibers. Avoid excessive toning that will result in undesirable rose to red tones.
13. Rinse in deionized water.
14. Place sections in 5% Sodium Thiosulfate for 1 minute.
15. Rinse in deionized water for 2 minutes.
16. Counterstain with Nuclear Fast Red for 5 minutes (optional).
17. Rinse well in deionized water.
18. Dehydrate in 95% alcohol for 1 minute.
19. Dehydrate sections in two changes of anhydrous (100%) alcohol for 1 minute each.
20. Clear in two to three changes of clearing reagent for 1 minute each and mount with synthetic mounting media.

Results

Reticulum Fibers – Black
Elastic – Black
Nerve Fibers – Black
Collagen – Lavender to Rose
Nuclei and Background – Red

Discussion

All staining reagents should be stored in a refrigerator at 2°C to 8°C. The Reticulum Fiber staining reagents are for "In Vitro" use only. Some of the reagents used in this kit are considered toxic. Refer to the Material Safety Data Sheet for Health and Safety Information. The Ammoniacal Silver reagent is stable but a precipitate may be noted; this will not affect reticular fiber staining. All other reagents are stable and should not form precipitate under recommended storage parameters. For best results it is recommended that the Ammoniacal Silver and Reducing Solution be discarded after use. The other components may be filtered back and reused. The Biological Stain Commission certified all dyes used in these formulations.

Technical Comments

As the alkalinity of the Ammoniacal Silver may result in tissue sections detaching from the slide, it is recommended that treated slides such as Thermo Scientific Bond-Rite slides, Poly-L-Lysine slides or a water bath additive be used. Plastic forceps should be used with all silver techniques to prevent formation of silver precipitate. Staining dishes should be thoroughly acid-washed and then rinsed with several changes of deionized water to eliminate the occurrence of precipitate that may interfere with the primary reaction. Prepare the working Ammoniacal Silver Solution just before use. Do not store prepared Ammoniacal Silver Solution; precipitate that may form may be unstable under certain storage parameters.

Probable Mode of Action

Potassium Permanganate is the oxidizing agent in this technique. It is followed by an Oxalic Acid treatment to remove the excess permanganate. This step serves to enhance the subsequent staining of the reticular fibers. A sensitizing step using Ferric Ammonium Sulfate impregnates the fibers creating a metal-organic bond that is replaced by silver during subsequent treatment with the Ammoniacal Silver. To develop the visible silver, a Reducing Solution of dilute formaldehyde is used. The tissue structures that have been impregnated with the silver solution will now appear brown to black. Gold Chloride is used to "tone" the sections producing better contrast and clarity as the gold reacts and combines with the reduced silver. Unreduced silver is removed via treatment with Sodium Thiosulfate Solution. An optional Nuclear Fast Red counterstain provides background staining as well as nuclear detail.

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. Lillie, R.D., H.J. Conn's Biological Stains. Williams & Wilkins, Baltimore, MD, 1972.
5. Luna, L.G., Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts. American Histolabs, Inc. Gaithersburg, MD, 1992.
6. Carson, F.L. Histotechnology: A Self-Instructional Text, 2nd Edition. ASCP Press, Chicago, 1997.

Order Information

Product	Size	Qty.	REF
Reticulin Silver Kit	1 Kit	1	87025
Oxalic Acid Solution	125ml	1	88045
Potassium Permanganate Solution	125ml	1	88046
Ferric Ammonium Sulfate Solution	125ml	1	88047
Reducing Solution	500ml	1	88048
Gold Chloride Solution (0.1%)	125ml	1	88026
Sodium Thiosulfate Solution	125ml	1	88025
Kernechtrot Nuclear Fast Red Stain Solution	125ml	1	88049
Silver Nitrate Solution (10%)	125ml	1	88050
Ammonium Hydroxide Solution	8ml	1	88051
Sodium Hydroxide Solution (3%)	125ml	1	88052

