Instructions for Use

Thermo Scientific Richard-Allan Scientific Chromaview – Advanced Testing

Reticulin Silver Stain

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-titrates).
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 caplin 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 reticulum fibers. Avoid excessive toning that will result in undesirable rose to red tones.
13. Rinse in deionized water.
14. Place sections in 5% Sodium Thioureact for 1 minute.
15. Rinse in deionized water for 2 minutes.
17. Rinse well in deionized water.
18. Detergent 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

Reticulin 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 Thioureact Solution. An optional Nuclear Fast Red counterstain provides background staining as well as nuclear detail.

References


Order Information

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Anatomical Pathology

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