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TECHNICAL DATA SHEET 876

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Reticulin Stain Kit

Used for the identification of reticulin fiber in tissue sections, most commonly the liver, kidney and spleen. Reticulin is a type III collagen found in the basement membrane of many organs and provides structural integrity. Reticular fibers characteristics can also aid in the diagnosis of certain tumors. In addition, changes in reticular pattern can be seen in some liver diseases. Reticulin Stain Kit is a metal impregnation technique where ammoniacal silver initially binds to the tissue component of interest. Then the reducing agent (formalin) produces a dark insoluble precipitate, enabling silver to be reduced into a visible state.

KIT COMPONENTS

Potassium Permanganate, 1% Aqueous Solution (250 or 500ml)
Oxalic Acid, 1% Aqueous Solution (250 or 500ml)
Ferric Ammonium Sulfate, 2.5% Aqueous Solution (250 or 500ml)
Silver Nitrate, 10% Aqueous Solution Store at 4°C (50 or 100ml)
Ammonium Hydroxide, 28-30% ACS (50 or 100ml)
Sodium Hydroxide, 3% Aqueous (50 or 100ml)
Formalin, 10% Aqueous Solution (250 or 500ml)
Gold Chloride, 0.2% Aqueous (250 or 500ml)
Sodium Thiosulfate, 5% Aqueous (250 or 500ml)
Nuclear Fast Red Stain Solution, 1% (250 or 500ml)

CONTROL Normal Liver

FIXATIVE 10% Formalin

TECHNIQUE Cut paraffin sections at 4m to 5m

EQUIPMENT Acid cleaned glassware and pipettes

TO MAKE SILVER SOLUTION:

- Place 5 ml of 10% silver nitrate solution into a beaker or flask, and add ammonium hydroxide, drop by drop, while stirring the solution continuously.
- Stir solution until precipitate that forms is fully dissolved.
- Next add 5 ml of 3% sodium hydroxide solution and cautiously redissolve the precipitate with ammonium hydroxide until only a faint cloudiness is observed.
- Afterwards, dilute the resulting solution to 50 ml with distilled water, and filter into a chemically clean Coplin jar.

PROCEDURE

- Deparaffinize in xylene and hydrate through graded alcohols to distilled water.
- Potassium permanganate solution, 5 minutes. (Oxidation)
- Wash in distilled water for 2 minutes.
- Bleach in oxalic acid until tissue is colorless.
- Wash in distilled water.
- Ferric Ammonium Sulfate for a minimum of 10 minutes. (Sensitization)
- Wash in running tap water, rinse in distilled, 3 changes.
- Silver nitrate solution, 7 dips, shake excess solution off slides. (Silver impregnation)
- Distilled water, 2 changes, 3 quick dips each.
- 10% formalin solution until gray black, 30 seconds to 2 minutes.
- Rinse well in distilled water. (Reduction/Developing)

- Tone in 0.2% Gold Chloride for a minimum of 1 minute. Color of impregnated component is changed from brown to black. Removes yellow background staining. (Toner)
- Rinse in distilled water.
- 5% Sodium Thiosulfate, 1 minute. (Removes unreduced silver)
- Wash in distilled water.
- Counterstain with Nuclear Fast Red Stain Solution for 5 minutes.
- Wash in running tap water.
- Dehydrate, clear, and coverslip.

RESULTS

Reticular Fibers - black
Nuclei - red

NOTES

- Use acid clean glassware.
- When making working silver solution, if over 30 drops of ammonium hydroxide are used to turn the solution, then the ammonium hydroxide is too old. Start over with fresh ammonium hydroxide.
- When adding the 3% sodium hydroxide solution to the silver solution it should turn black, if not make fresh sodium hydroxide.
- Because of the alkalinity of the solution, it may cause some tissues to fall off the slides. Use charged slides to prevent section loss.
- The water rinse in step 9 is critical for good reticulin demonstration. If the wash is prolonged, the staining of the reticulin will be reduced, and if it is insufficient, there will be excessive background staining.

REFERENCE

Bancroft J, Stevens A, Theory and Practice of Histological Techniques, 2nd Ed, 1982, pp 142-143, Churchill Livingstone, NY
Carson F, Histotechnology: A Self-Instructional Text, 11997, pp 143-145, ASCP, Chicago
Crookham, J, Dapson, R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech
Lillie, R.D. 1977 H.J. Conn's Biological Stains, 9th edition Williams and Wilkins

Please refer to the MSDS for chemical and safety information

TO ORDER

Cat. #	Description
25094-250	Reticulin Stain Kit - 250 ml kit (components A-J)
25094-500	Reticulin Stain Kit - 500 ml kit (components A-J)

Replacement kit components available; please contact customer service for pricing.

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