COLLOIDAL IRON - MUCOPOLYSACCHARIDES

PURPOSE: To demonstrate carboxylated and sulfated mucopolysaccharides and glycoproteins.

PRINCIPLE: Colloidal ferric ions are, at low pH, absorbed principally by carboxylated and sulfated mucosubstances. The excess reagent is washed out and the classic Prussian blue reaction (iron stain) is used to demonstrate iron bound to the tissue.

CONTROL: Use mucin control, small intestine

FIXATION: 10% formalin, avoid chromate fixatives.

TECHNIQUE: Cut paraffin sections at 4m.

EQUIPMENT: Hot plate, acid-clean glassware: flasks, cylinders, and coplin jars, filter paper.

REAGENTS:

Müller Colloidal Iron
Stock Solution:

Boiling distilled water	250.0 ml
29% ferric chloride-fresh	4.4 ml

Keep the water boiling and stirring when adding ferric chloride. Stir until solution becomes dark red. Remove from heat, cool. Store in clean brown bottle, label with date and initial. Solution is stable for 6 months.

Working Solution:

Stock colloidal	10.0 ml
Distilled water	18.0 ml
Acetic acid	12.0 ml

Prepare fresh, discard after use.

CAUTION: Avoid contact and inhalation.

12% Acetic Acid:

Glacial acetic acid	24.0 ml
Distilled water	176.0 ml

CAUTION: Corrosive acid.

Ferrocyanide-Hydrochloric Acid Working Solution (Iron Stain)

5% Potassium ferrocyanide	25.0 ml
5% Hydrochloric acid	25.0 ml

Mix right before use, use acid clean glassware. Discard after use.

CAUTION: Corrosive acid.

Nuclear-Fast Red:
See Iron Stain

COLLOIDAL IRON - MUCOPOLYSACCHARIDES

SAFETY: Wear gloves, goggles and lab coat. Boil solution in fume hood. Add acid to water. Avoid contact and inhalation.

Acetic acid: Irritating to eyes, skin and respiratory system, target organ effects on respiratory system. Corrosive.

Ferric chloride: corrosive to metal. Causes GI problems on ingestion.

PROCEDURE:

1. Deparaffinize and hydrate sections to distilled water.
2. Rinse slides in 12% acetic acid.
3. Working colloidal iron solution for 1 hour.
4. Rinse in 12% acetic acid, 3 changes, 3 minutes each.
5. Ferrocyanide-hydrochloric acid solution for 20 minutes.
6. Wash in running tap water for 5 minutes.
7. Counterstain with Nuclear-fast red for 5 minutes.
8. Wash in running tap water.
9. Dehydrate, clear and mount.

RESULTS:

Acid mucopolysaccharides and sialomucins	deep blue
Nuclei	pink-red
Cytoplasm	pink

REFERENCE:

F.CARSON, HISTOTECHNOLOGY: A SELF-INSTRUCTIONAL TEXT, PP 127-129, 1991, ASCP PRESS

Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared:_____By: _____

Approved:_____By:_____

PROCEDURE CARD

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TECH:_____

DATE:_____

EXPIRATION:_____

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Distilled water	176.0 ml

Mix well, label with inital and date. Solution is stable for 6 months.

CAUTION: Corrosive acid.

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DATE:_____

EXPIRATION:_____