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MOVAT Pentachrome Staining

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

The following table describes the staining color for various structures.

Results:	Nuclei and Elastic Fibers	Black
	Ground Substance and Mucin	Blue
	Muscle	Red
	Collagen and Reticular Fibers	Yellow
	Fibrinoid and Fibrin	Intense Red

Diabetic Complications:



Cardiovascular



Nephropathy



Neuropathy



Retinopathy



Uropathy

Materials

Reagent Preparation:

Alkaline Alcohol

95% ETOH	45 ml
conc. Ammonium Hydroxide	5 ml

Bouins's Solution

Picric Acid, saturated aqueous	75 ml
Formaldehyde 37-40%	25 ml
Glacial Acetic Acid	5 ml

1% Alcian Blue

Alcian Blue	1 gm
Distilled Water	100 ml

STOCK Weigert's Hematoxylin

2% Alcoholic Hematoxylin

Hematoxylin	10 gm
95% ETOH	500 ml

Ferric Chloride

Ferric Chloride Hexahydrate	12.4 gm
Distilled Water	500 ml
HCL, conc	5 ml

Iodine Solution

Iodine	10 gm
Potassium Iodide	20 gm
Distilled Water	500 ml

*Dissolve the Potassium Iodide in a small amount of the distilled water. Add the iodine, when completely dissolved add the remainder of the distilled water.

WORKING Weigert's Solution

2% Hematoxylin	30 ml
Ferric Chloride	20 ml
Iodine Solution	10 ml

*Mix in order, use immediately. Discard after use.

STOCK Crocein Scarlet

Crocein Scarlet MOO 3B	1.0 gm
Distilled Water	98.5 ml
Conc. Glacial Acetic Acid	0.5 ml

STOCK Acid Fuchsin

Acid Fuchsin	0.1 gm
Distilled Water	99.5 ml
Conc. Glacial Acetic Acid	0.5 ml

WORKING Crocein Scarlet/Acid Fuchsin

Stock Crocein Scarlet	40 ml
Stock Acid Fuchsin	10 ml

5% Phosphotungstic Acid

Phosphotungstic Acid	5 gm
Distilled Water	100 ml

1% Glacial Acetic Acid

Conc. Glacial Acetic Acid	1 ml
Distilled water	99 ml

Alcoholic Saffron

Saffron	6 gm
Absolute Alcohol	100 ml

* Store in airtight bottles in 55-60 degree oven for 48 hrs to extract saffron (Do not add additional alcohol to the solution as it evaporates because it will weaken the staining). Cool the extract to room temperature, filter through a coarse filter paper in a covered funnel and store in the filtrate in a dark tightly stoppered container.

5% Sodium Thiosulfate

Sodium Thiosulfate	5 gm
Distilled Water	100 ml

0.1% Sirius Red Solution

Sirius Red Dye	0.1 gm
Saturated Picric Acid	100 ml

*Store in refrigerator, stable for 4-6 months.

Troubleshooting

- 1 Deparaffinize and hydrate to distilled water
- 2 Preheat Alkaline Alcohol in 60 degree oven
- 3 Mordant in Bouins in microwave, covered, at Med power for 45 seconds. Let sit for 10 mins. (Do Not Dump)
- 4 Rinse in cold running water for 10 minutes
- 5 Sodium Thiosulfate for 5 minutes, wash for 5 minutes under cold running water
- 6 Stain in 1% Alcian Blue for 20 minutes (Do Not Dump)
- 7 Cold running water for 2-5 minutes
- 8 Place in preheated Alkaline Alcohol for 10 minutes. (in oven)
- 9 Cold running water for 2-5 minutes
- 10 Place in Movat's Weigerts for 60 minutes (Do not Microwave)
- 11 Wash briefly in cold running water; rinse well in distilled water
- 12 Place in Crocein Scarlet/Acid Fuchsin for 1 min
- 13 Rinse in distilled water



- 14 Place in 5% Phosphotungstic Acid for 5 min
- 15 Transfer directly to 1% Acetic Acid for 5 minutes
- 16 Rinse well in distilled water
- 17 Dehydrate in 1 change of 95% ETOH, 2 changes of 100% ETOH 1 minute each
- 18 Alcoholic Saffron for 20 minutes (Do Not Dump)
- 19 Rinse in 2 changes of 100% ETOH for 1 minute each
- 20 Clear in histoclear (xylene) and mount