MASSONS TRICHROME STAINING PROTOCOL FOR TUBULOINTERSTITIAL DISEASE

Version: 1
Replaced by version: N/A
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Summary: Massons Trichrome staining is used for detection of collagen fibers for tubulointerstitial fibrosis on formalin-fixed paraffin-embedded kidney tissue section. The collagen fibers will be stained blue, the nuclei black, and the background is stained red.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Weigert's Iron Hematoxylin Solution Set	Sigma-Aldrich	Solution A HT107, Solution B HT109
Bouins solution	Sigma-Aldrich	HT10132
Biebrich Scarlet-Acid Fuchsin solution	Sigma-Aldrich	HT151
Phosphomolybdic Acid solution	Sigma-Aldrich	HT153
Phosphotungstic Acid solution	Sigma-Aldrich	HT152
Aniline Blue solution	Sigma-Aldrich	B8563
Acetic acid, 1%	Fisher Scientific	A38-212
Xylene	Fisher Scientific	X3P-1GAL
Ethanol (EtOH) 200 Proof	Decon-Laboratories Inc	2701
Per mount	Thermo Scientific	8312-4
Cover slip	Fisher Scientific	12-542-B

Protocol:

WARNING HAZARDOUS CONDITION WARNED AGAINST. This comment describes a hazardous condition to which the technician may be exposed in the performance of this protocol. It also contains directions on how to avoid or minimize the danger. Warnings are always and only used for personnel safety, and precedes the first step that will expose the technician to the hazard.

1) Wash 2x for 5 minutes in Xylene
2) Wash 2x for 3 minutes in 100% EtOH
3) Wash 1x for 2 minutes in 95% EtOH
4) Wash 1x for 1 minutes in 70% EtOH
5) Wash in running tap water for 3 minutes
6) Place in Bouins for one hour in 55 -56 degree oven or overnight at room temperature if needed.
7) Wash well in running water for 5-10 minutes or until yellow is gone.
8) Stain in Weigerts Hematoxylin for 10-20 minutes (usually 10 minutes).
9) Wash well in running water for 10 minutes.
10) Rinse distilled water.
11) Place slides in Biebrich Scarlet-Acid Fuchsin for 5-10 minutes.
12) Rinse in distilled water.
13) Place slides in the Phosphomolybdic-Phosphotungstic Acid Solution in a plastic coplin jar for 10-15 minutes or until collagen fibers are not red.
14) Place slides in Aniline Blue solution for 10 minutes.
15) Rinse in distilled water.
16) Place in 1% Acetic acid for 3 minutes.
17) Wash 2x for 3 minutes in 100% EtOH
18) Wash 1x for 2 minutes in 95% EtOH
19) Wash 2x for 5 minutes in Xylene
20) Mount slides with mounting medium- 1 drop
21) Insert Coverslip carefully, avoid bubble

Reagent Preparation:

Reagent 1: 1%Acetic Acid
Reagents and Materials: Acetic Acid and distilled water
Procedure: Mix 1ml of Glacial acetic acid with 99ml of distilled Water (use flow hood)
Checklist: N/A