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U Michigan - Massons Trichrome staining V.2 [↗](#)

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1 Works for me [dx.doi.org/10.17504/protocols.io.56ug9ew](https://doi.org/10.17504/protocols.io.56ug9ew)

Mouse Metabolic Phenotyping Centers
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ABSTRACT

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.

EXTERNAL LINK

<https://mmpc.org/shared/document.aspx?id=311&docType=Protocol>

MATERIALS

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

MATERIALS TEXT

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

Note:

Sigma-Aldrich, [RRID:SCR_008988](#)

Fisher Scientific, [RRID:SCR_008452](#)

- 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



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