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Working

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

Jeff Hodgkin<sup>1</sup>

<sup>1</sup>University of Michigan - Ann Arbor

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Mouse Metabolic Phenotyping Centers  
Tech. support email: [info@mmpc.org](mailto:info@mmpc.org)

Lili Liang

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

### 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](#)

#### SAFETY WARNINGS

**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



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