



Aug 07,
2019

U Michigan - Sirius Red staining V.2 [↗](#)

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1 Works for me [dx.doi.org/10.17504/protocols.io.56tg9en](https://doi.org/10.17504/protocols.io.56tg9en)

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ABSTRACT

Summary:

Picrosirius red staining (also called Sirius red staining) highlights fibrosis by staining collagen (collagen 1 and III fibers) fibers in paraffin-embedded kidney tissue sections. Sirius red stained collagen appears red by light microscopy. When polarized, the birefringence is highly specific for collagen. The larger collagen fibers are bright yellow or orange, and the thinner ones, including reticular fibers, are green.

EXTERNAL LINK

<https://mmpc.org/shared/document.aspx?id=313&docType=Protocol>

MATERIALS

NAME	CATALOG #	VENDOR
Sirius Red (Direct Red 80)	365548	Sigma Aldrich
Picric acid	5860-1	Fisher Scientific
Acidified Water		
Hematoxylin 2	7231	Richard-Allan Scientific
Acetic acid Glacial	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: Picro-Sirius Red Solution:

Reagents and Materials: Sirius Red (Direct Red 80) powder, saturated aqueous solution of picric acid

Procedure: Mix 0.5g Sirius red powder with 500 ml of saturated aqueous solution of picric acid. This solution is stable for at least 3 years and can be re-used

Reagent 2: Acidified Water

Reagents and Materials: Acetic acid and distilled water (milliQ water)

Procedure: Mix 5 ml of acetic acid with 1000 ml water.

Note:

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

Fisher Scientific, [RRID:SCR_008452](#)

- 1 Wash 2x for 5 min in Xylene
- 2 Wash 2x for 3 min in 100% EtOH
- 3 Wash 1x for 2 min in 95% EtOH
- 4 Wash 1x for 2 min in 80% EtOH
- 5 Wash 1x for 1 min in 70% EtOH
- 6 Wash in running tap water for 3 min
- 7 Incubate in hematoxylin for 8 min.
- 8 Wash in running tap water for 10 min
- 9 Incubate for 60 min in Picrosirius red solution (do not shorten this time)
- 10 Wash 2x for 5 min in acidified H₂O (Change after each wash)
- 11 Wash 1x for 1 min in 70% EtOH
- 12 Wash 1x for 1 min in 80% EtOH
- 13 Wash 1x for 1 min in 95% EtOH
- 14 Wash 1x for 1 min in 100% EtOH
- 15 Wash 2x for 3 min in Xylene

16 Mount slides with mounting medium - 1 drop

17 Insert cover glass carefully, avoid bubble



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