



PICRO SIRIUS RED STAIN FOR TUBULOINTERSTITIAL DISEASE

Version: 1

Replaced by version: N/A

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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.

Reagents and Materials:

| Reagent/Material | Vendor | Stock Number |
|----------------------------|---------------------------------------|--------------|
| Sirius Red (Direct Red 80) | Sigma Aldrich | 365548 |
| Picric acid | Ricca Chemical from Fisher Scientific | 5860-1 |
| Acidified Water | N/A | N/A |
| Hematoxylin 2 | Richard-Allan Scientific | 7231 |
| Acetic acid, Glacial | 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 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

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

Checklist: N/A

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.

Checklist: N/A