Picro-Sirius Red Histochemistry

Reagents

- Direct Red 80 (Sigma, cat. #365548-5G)
- 1.3% Saturated Picric Acid Solution (Sigma, cat. #P6744-1GAL)
- Weigert's Iron Hematoxylin Solution A (Harleco, cat. #15204-220)
- Weigert's Iron Hematoxylin Solution B (Harleco, cat. #15204-222)
- Glacial Acetic Acid (Fisher Scientific, cat. #A38-500)

Preparation of Reagents

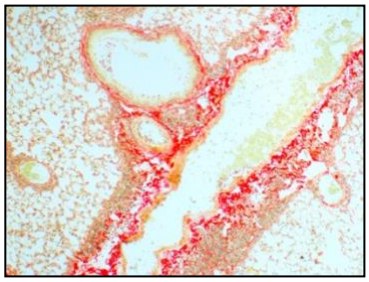
1. Weigert's Iron Hematoxylin working solution
    a. Mix Solution A and B in 1:1 ratio (*can be used for up to 2 weeks*)
2. 0.02% Picro-Sirius Red
    a. 0.1 g of Direct Red 80 in 500 mL of 1.3% saturated picric acid solution (*can be used for up to 2-3 years*)
3. 0.5% Glacial Acetic Acid (50 mL glacial acetic acid in 1 L Milli-Q water)

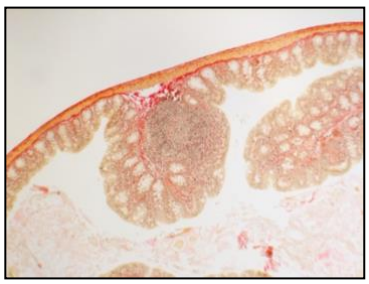
Procedure

1. Deparaffinize paraffin sections and place in slides Weigert's Iron Hematoxylin for *8 min*
2. Move the slides to running tap water, rinse for *10 min*
3. Immerse slides in 0.02% Picro-Sirius Red for *1 h*
4. Immerse slides ***twice*** in 0.5% Glacial Acetic Acid/Milli-Q water – *10 dips* ***each***
5. Physically shake slides to remove the remaining water
6. Dehydrate (100% EtOH only) and clear in xylene in the fume hood

Notes

- Control tissue: colon, lung
- Expected appearance: collagen – red, nuclei – black, everything else – yellow
- Works better on mouse tissue compared to Masson's Trichrome
- Can differentiate between collagen fibers via polarizer (larger fibers – yellow/orange, thinner fibers – green)





Wildtype mouse lung (left) and wildtype mouse proximal colon (right) with highlighted red collagen fibers, which can also be visualized using polarized light