Masson's Trichrome

Reagents

- Bouin's Solution (Ricca Chemical, cat. #1120-32)
- Weigert's Iron Hematoxylin Solution A (Harleco, cat. #15204-220)
- Weigert's Iron Hematoxylin Solution B (Harleco, cat. #15204-222)
- Biebrich Scarlet-Acid Fuchsin Solution (Sigma, cat. #HT15-1)
- Phosphotungstic Acid Solution or PTA (Sigma, cat. #HT15-2)
- Phosphomolybdic Acid Solution or PMA (Sigma, cat. #HT15-3)
- Aniline Blue Solution (Sigma, cat. #HT15-4)
- 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. Fresh Phosphomolybdic/Phosphotungstic Acid working solution (PMA/PTA solution)
    a. Mix PMA, PTA, and Milli-Q water in 1:1:2 ratio
3. 1% Glacial acetic acid (100 mL glacial acetic acid in 1 L Milli-Q water)

Procedure

1. Deparaffinize and rehydrate sections on slides
2. Place slides in a container of Bouin's solution **in the fume hood** and incubate *EITHER* overnight at room temperature *OR* for 1 h at 60°C
3. Place slides in a slide rack under running tap water until the yellow color is washed away
4. Place slides in a tub of Weigert's Iron Hematoxylin solution for *5 min*
5. Wash slides under running tap water for *5 min*
6. Rinse slides in DI water – *10 dips*
7. Place slides in Biebrich Scarlet-Acid Fuchsin solution for *5 min*
8. Move slides to be rinsed ***3 times*** in DI water – at least *10 dips* ***each***
9. Place slides in Working PMA/PTA Solution for *5 min*
10. Place slides in "Old" Aniline Blue for *1 dip* (to prevent "contamination" of Aniline Blue with leftover PMA/PTA)
11. Place slides in ***new*** Aniline Blue for *5 min*
12. Place slides in 1% Glacial Acetic Acid solution for *2 min*
13. Move slides to be rinsed ***3 times*** in DI water – *10 dips* ***each***
14. Dehydrate and clear in xylene **in the fume hood** and mount using permanent mounting medium

Notes

- Control tissue: colon, lung
- Expected appearance: collagen, blue; nuclei, black; muscle fibers, red; cytoplasm, red; everything else, yellow
- This histochemical stain works best when using paraffin sections

Example Images

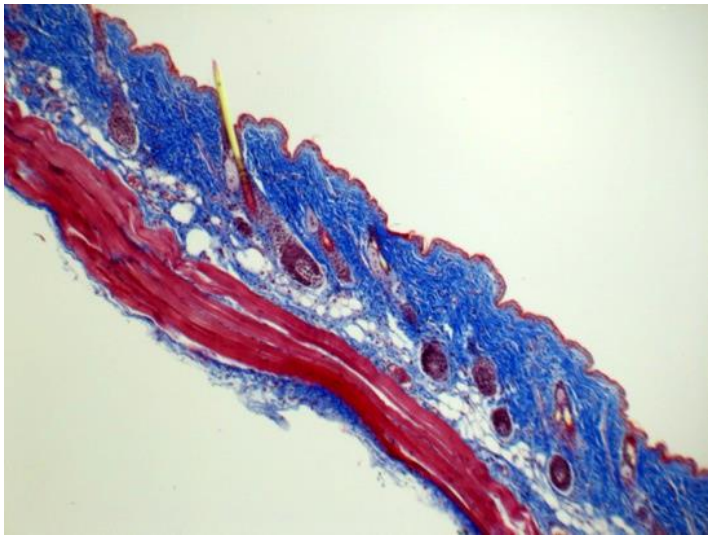

Figure 1. Mouse skin stained with Masson's Trichrome.

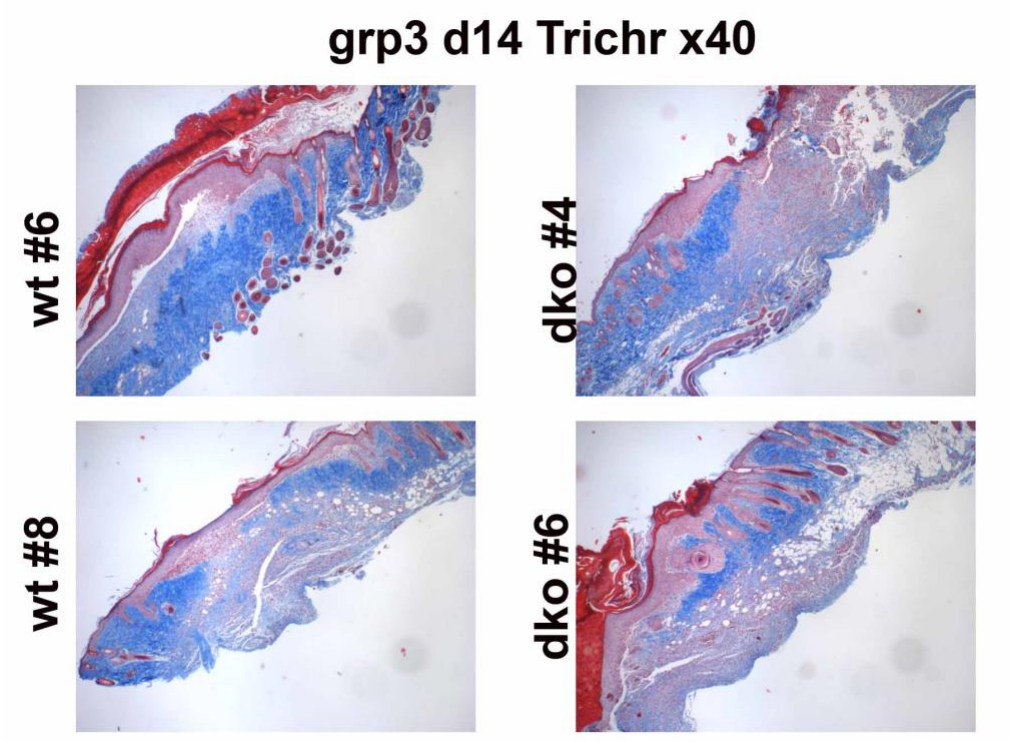

Figure 2. Paraffin sections of mouse skin stained with Masson's Trichrome. Blue staining of collagen in the dermis is used to quantify the scarring that occurs as part of the healing process after a wound.