

# Mallory's Tetrachrome Staining Of Mouse Sections

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Adapted from an original protocol by P. Chambon

This procedure allows the visualization of cellular structures as follows:

- nucleus will be stained in black
- cytoplasm will be stained in purple-pink
- collagene fibers will be stained in blue

Note: This protocol is used for embryos 14.5 days post coitum and older

## **Dissection**

- Dissect in 1x PBS
- Punch holes in the skin of the embryos to allow a better penetration of the fixative

## **Fixation and Decalcification**

- Fix in Bouin's solution for 4-8 days
- Decalcify in Jenkin's for 48 hours replacing with fresh solution at least three times. Use at least an amount of 50x the volume of the embryo to allow a good decalcification

## **Embedding and sectioning**

- Wash 2x in 95% EtoH for 1 hour
- Wash 1x in 95% EtoH for 12 hours
- Wash 1 day in 100% EtoH replacing with fresh solution at least three times
- Wash 1 day in toluene replacing with fresh solution at least three times
- Wash 1 day in filtered paraffin (Mc Cormick scientific cat# 502004) in an oven at 75°C replacing with fresh solution at least three times
- Wash 1 hour in paraffin inside a vacuum oven at 75°C to prevent paraffin from solidifying
- Embed embryos in fresh paraffin
- Allow the blocks to solidify at least 24 hours
- Cut 7µm thick sections using a microtome

## **Mallory's staining**

\*It is recommended to do a test run to adjust staining times in Groat solution, Acid Fuschine and Aniline solution for your specific sample

- Immerse sections 2x 5 minutes in toluene
- Immerse sections 2x 5 minutes in 100% EtOH
- Immerse sections 1x 5 minutes 95% EtOH
- Immerse sections 1x 5 minutes in 70% EtOH
- Immerse sections 1x 5 minutes\* in Groat solution
- Immerse sections 1x 5 minutes in tap water
- Immerse sections 1x 3 minutes\* in Acid Fuschine
- Immerse sections 1x 30 minutes\* in Aniline solution
- Immerse sections 3x 1 minutes in 95% EtOH
- Immerse sections 2x 3 minutes in 100% EtOH
- Immerse sections 2x 5 minutes in toluene
- Mount in permount

## **Solutions**

**EtOH:** EtOH anhydrous, histological grade, Fisher cat# A405P-4 is used for this protocol

### **Bouin's solution**

75 ml saturated picric acid  
25 ml 40% formaldehyde  
5 ml glacial acetic acid

### **Jenkin's solution**

40 ml 6N HCl  
30 ml glacial acetic acid  
100 ml chloroform  
730 ml 100% EtOH  
100 ml dH<sub>2</sub>O

### **Groat solution**

1g sulphate of ammonium and iron III in 50 ml dH<sub>2</sub>O  
Add 0.8 ml sulfuric acid  
50 ml 95% EtOH  
0.5g hematoxyline  
18mg potassium permanganate  
dH<sub>2</sub>O QSP 100 ml

**Acid Fuschine:** 0.5% in dH<sub>2</sub>O

### **Aniline solution**

4g orange G  
2g acid phosphotungstic  
1g aniline blue  
dH<sub>2</sub>O QSP 200 ml