

Hematoxylin & Eosin (HE) staining - Hiroki Kuroda

(Sectioning)

Time courses are different in sample types and stages.

(Time1/Time2/Time3/Time4) means (stages less than 20/stages 20-30/stages greater than 30/explants).

- Fix sample in Bouin's solution at RT (5h/2h/2h/30m)
- 70% EtOH (3h/1h/1h/10m) Note. You can store sample in 70% EtOH forever at 4°C
- 100% EtOH (2h/30m/30m/10m)
- 100% EtOH (2h/1h/30m/10m)
- Isopropanol (2h/1h/30m/10m)
- Xylene (overnight/5h/30m/10m)
- Xylene (1h/1h/15m/5m)
- Paraffin at 60°C (overnight/2h/30m/10m)
- Paraffin at 60°C (2h/1h/15m/5m)
- embed
- Section

(Staining)

- Xylene for 5 min
- Xylene for 5 min
- 100% EtOH for 1 min
- 100% EtOH for 1 min
- 70% EtOH for 1 min
- Tap water for 5 min
- Hematoxylin solution for 1min
- Wash with tap water for a while
- Slowly run tap water for 15 min
- 50-60°C tap water for 15 min
- Eosin solution for 1 min
- Tap water for a while
- 70% EtOH for a while
- 100% EtOH for a while
- 100% EtOH for 2 min
- Xylene for 2 min
- Xylene until mounting sample with a cover slide



Bouin's Solution (42ml)

Picric acid 30 ml, Acetic acid 10 ml, 30-40% formaldehyde 2ml
Better if used fresh but you can keep at 4°C for two weeks

Hematoxylin solution

3-fold dilution of liquid Hematoxylin Stain Gill's Formulation #3 (Fisher Scientific: Cat# CS402-1D) with water.
Or make the following solution:
0.2 % Hematoxylin, 0.02% NaLo₃ (Sodium Lodate), 3.5% Al₂(SO₄)₃, 2% Glacial Acetic Acid, 8% Ethylene Glycol, with water.

Eosin solution (1L)

Eosin Y 2.5g, 10% Acetic Acid 2.5 ml in 1L of 60% EtOH
Note. Store at RT in the dark