

H&E (Haematoxylin and Eosin) Staining for Frozen Tissue Sections

1. Air dry sections for several minutes to remove moisture.
2. Stain with filtered **0.1% Mayers Hematoxylin** (Sigma; MHS-16) for 10 minutes in a 50 ml conical tube. **If Hamatoxylin is stored in 50 ml tube it should be wrapped in foil.
3. In a Coplin jar, Rinse in cool running ddH₂O for 5 minutes.
4. Dip in **0.5% Eosin** (1.5g dissolved in 300ml of 95% EtOH) 12 times.
5. Dip in distilled H₂O until the eosin stops streaking.—Don't overwash!
6. Dip in 50% EtOH 10 times
7. Dip in 70% EtOH 10 times
8. Equilibrate in 95% EtOH for 30 seconds.
9. Equilibrate in 100% EtOH for 1 minute.
10. Dip in Xylene several times.
11. Clean slide off with a kimwipe; mount and coverslip with Cytoseal XYL (Stephens Scientific; cat# 8312-4)

H&E Staining for Parrafin Sections

1. Melt paraffin off slides @ 65° C for 20 minutes.
2. Treat with Xylene twice for 10 minutes each.
3. Treat with 100% EtOH twice for 5 minutes each.
4. Air dry slides
5. Stain with filtered 0.1% Mayers Hematoxylin for 10 minutes in a Coplin jar.
6. Rinse in cool running ddH₂O for 5 minutes
7. Stain with Eosin (0.5% in 95%EtOH) 12 times.
8. Dip in distilled H₂O until the eosin stops streaking.—Don't overwash!
9. Dip in 50% EtOH 10 times
10. Dip in 70% EtOH 10 times
11. Equilibrate in 95% EtOH for 30 seconds.
12. Equilibrate in 100% EtOH for 1 minute.
12. Dip in Xylene several times.
13. Clean slide off with a kimwipe; mount and coverslip with Cytoseal XYL