

Hematoxylin & Eosin Staining of Paraffin Sections
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1. Deparaffinize sections in xylene for 2x 3 min
2. Rehydrate sections in 100% Ethanol (EtOH) for 2x 2 min.
3. Put the slides through 90% and 70% EtOH for 2x 2 min.
4. 5 min in dH₂O.
5. Stain with Hematoxylin QS, 3 min
6. Rinse in deionized H₂O, 1 min
7. 5 min in tap water (for the staining to develop)
8. Dip the slides 2-3 times in Acid EtOH (to destain)
9. Rinse in tap water twice, 1 min each
10. Rinse in deionized H₂O for 2 min (can leave overnight at this stage)
11. Eosin solution, 30 sec (up to 45 sec for an older batch of Eosin)
12. 90% EtOH for 2x 2 min
13. 100% EtOH for 2x 2 min
14. Xylene, 2x 3 min
15. Mount cover slip with Permount or other Xylene based adhesives (e.g Eukitt mounting media, Electron Microscopy Sciences, Cat#15320).

Solutions

0.5% Acid Ethanol: 1 ml concentrated HCl + 50 ml 70% ethanol.

Hematoxylin QS (Vector Labs, H3404)

Eosin Y stock solution (1% w/v): dissolve 1 g Eosin Y in 100 mL dH₂O

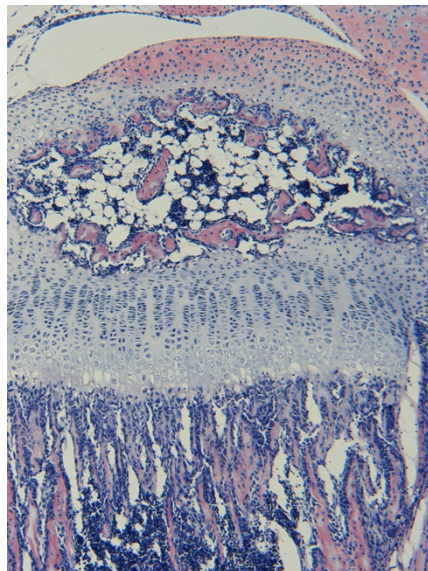
Eosin Y working solution (0.1% w/v in 90% EtOH):

Eosin Y stock solution, 20 mL

90% EtOH, 200 ml

Glacial acetic acid, 1 ml

Results:



H&E staining of P17
mouse proximal
tibia

Nucleus = black
Cytoplasm = pink