

HDBR Fact Sheet

Haematoxylin and Eosin Staining

Tissue sectioned are H&E stained at regular intervals following the protocol below.

- 1. Filter haematoxylin to remove the oxidised film from the solution.
- 2. Prior to staining a batch of slides, perform a test slide to establish the appropriate staining times required for that samples. If necessary, this slide can be destained prior to staining the whole batch of slides.
- 3. Place slides containing the sectioned tissue into a metal rack. Plastic racks are not compatible with xylene and will not fit properly in the glass troughs.
- 4. Dewax racked slides in xylene pots in fume hood 5 mins.
- 5. Rehydrate slides through graded ethanols (100%, 100%, 95%, 70%, 50%) each fort 3 minutes.
- 6. Rinse in running tap water.
- 7. Place in Harris' haematoxylin for 30 seconds if solution is fresh, 1 minute or longer with older solution. Time is determined using the test slide.
- 8. Rinse in running tap water.
- 9. 'Blue' in Scot's Tap Water substitute. The sections will look dark blue.
- 10. Rinse in running tap water.
- 11. Differentiate in 1% acid alcohol until the sections are a moderate peach/orange colour.
- 12. Rinse in running tap water.
- 13. Blue in Scot's Tap Water substitute.
- 14. Check nuclear staining under microscope, repeat differentiation if necessary (if the nuclei are too dark).
- 15. Place in Eosin 60 seconds or longer (exact time determined by test slide). The pink should not be too bright.
- 16. Rinse in running tap water. Check the slides under the microscope to see the balance of blue and pink.
- 17. Dehydrate quickly (~3 seconds each) through graded ethanols (50%, 70%, 95%, 100%,100%). Eosin will elute into water/alcohol, so if the eosin is too strong, the dehydration can be slightly slower record the times.

- 18. Clear in two changes of xylene.
- 19. Mount in DPX.

Solutions used:

Acid Alcohol:

1ml of conc. HCL in 1L of 70% ethanol

Scott's Tap Water Substitute:

20g Sodium Hydrogen Carbonate3.5g Magnesium Sulphate1L of distilled water

Harris Haematoxylin (Sigma Aldrich) HS128