HDBR
HUMAN DEVELOPMENTAL BIOLOGY RESOURCE

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 for 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.

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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 Carbonate
3.5g Magnesium Sulphate
1L of distilled water

Harris Haematoxylin (Sigma Aldrich) HS128