



Histology Services Protocols

H&E Staining Protocol (Surgipath)

1. Xylene	3 minutes
2. Xylene	3 minutes
3. Xylene	3 minutes
4. 100% Anhydrous Alcohol	1 minute
5. 100% Anhydrous Alcohol	1 minute
6. 100% Anhydrous Alcohol	1 minute
7. 80% Alcohol	1 minute
8. Tap water rinse	30 seconds to 1 minute
9. Rinse in deionized water	30 seconds to 1 minute
10. Hematoxylin 560	1 to 5 minutes
11. Tap water rinse	30 seconds to 1 minute
12. Define	30 to 90 seconds
13. Tap water rinse	30 seconds to 1 minute
14. Blue buffer 8	30 seconds to 1 minute
15. Tap water rinse	30 seconds to 1 minute
16. 80% Alcohol	1 minute
17. Alcoholic Eosin Y	30 to 90 seconds
18. 100% Anhydrous Alcohol	1 minute
19. 100% Anhydrous Alcohol	1 minute
20. 100% Anhydrous Alcohol	1 minute
21. Xylene	3 minute
22. Xylene	3 minute
23. Xylene	3 minute

24. Cover-slip in a miscible mounting media of the clearing agent.

Expected Results: (Stain run time is approximately 28-34 minutes.)

Stained sections should demonstrate deep-blue nuclei with a clearly defined heterochromatin pattern. Nuclear staining intensity is controlled by the amount of time the sections are exposed to Hematoxylin 560 during the staining procedure.

Recommended immersion time in Hematoxylin 560 is 1 to 5 minutes. An increase in staining time in the Hematoxylin will produce a more intense nuclear chromatin stain. Cytoplasm, muscle fibers and collagen should demonstrate varying shades of pink to red. A 30 second rinse of 80% to 70% alcohol may increase cytoplasm intensity. The exposure to Define increases cell definition, eliminates background staining, and minimizes the absorption of stained mucin.

Hematoxylin 560™, Blue Buffer 8™, Define™, Alcoholic Eosin Y 515™ and Eosin Trichrome 515™.