

1 Day ABC PROCEDURE FOR PARAFFIN SECTIONS

DAY 1

1. Immerse sections in 2X Xylene 5 min each
2. Immerse sections in descending EtOH series (100%, 95%, 80%, 70%) 1 min each
3. Immerse sections in dH₂O 1 min
4. Immerse in freshly prepared Methanol/H₂O₂ (150ml Methanol + 30ml stock 30% H₂O₂) 30min
5. Wash sections in tap water 5min
6. Pretreatment if necessary
7. Immerse in 0.1M Tris buffer (pH7.6) 5min
8. Immerse in 0.1M Tris/2%FBS bath 5min
9. Wipe excess fluid from around tissue; apply 100ul of Primary Antibody to section
10. Incubate at RT in humidified chamber 30 min
11. Rinse off Ab from tissue using 0.1M Tris; carefully direct spray from wash bottle around tissue, **NOT** directly on it
12. Immerse in 0.1M Tris 5min
13. Immerse in 0.1M Tris/2%FBS 5min
14. Apply 100ul biotinylated linking Ab (Vector) to section as in step 8 (stock in 4°C Histochem use at 1:200 dilution)
15. Incubate at room temp. in humidified chamber for 30min.
16. Rinse off Link Ab as in step 10
17. Immerse in 0.1M Tris 5min
18. Immerse in 0.1M Tris/2%FBS 5min
19. Add equal amounts of **B** to **A** in Tris/FBS at 1:200 dilution from the Vector ABC kit (4°C Histochem Frig) Vortex and wait 15min for solution to complex
20. Apply 100ul of ABC to section as in step 9
21. Incubate at room temp. in humidified chamber for 30min.
22. Rinse off ABC as in step 10
23. Immerse in 0.1M Tris 5min
24. Prepare DAB: cover work area with lab mat to absorb spills – Wear gloves and lab coat: DAKO (K3468) 1drop substrate per 1ml buffer.
25. Remove slides from Tris bath; place directly into incubation chamber and apply DAB to tissue with disposable pipette, making sure to cover entire tissue section.
26. After developing for 10 min, rinse off DAB as in step 10
27. Immerse 2X in ddH₂O 1 min each.
28. Filter Harris Hematoxylin into a clear boat.
29. Immerse sections in Hematoxylin for 20sec.
30. Immediately rinse in running tap water for 15 min.
31. Dehydrate sections in ascending series of EtOH (70%, 80%, 95%, 100%, 100%) 1min each
32. Clear in 2 changes of Xylene 5 min each
33. Coverslip with Cytoseal

Notes

- a) discard all Tris washes after each use
- b) re-use 0.1M Tris/2% FBS blocking baths up to 1 week, refrigerate at 4°C
- c) use 0.1M Tris/2% FBS to dilute Ab, biotinylated Linking Ab, and ABC
- d) use bleach to decontaminate everything contacted by DAB