

PATHOLOGY CORE

Overnight IF Procedure For Parafin 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. Pretreat if necessary
5. Immerse in 0.1M Tris buffer (pH7.6) 5 min
6. Immerse in 0.1M Tris/2%FBS bath 5 min
7. Wipe excess fluid from around tissue; apply 100ul-200ul of Primary Antibody to section
8. Incubate at 4°C in humidified chamber overnight (16-20 hrs)

DAY 2

9. Rinse off Ab from tissue using 0.1M Tris; carefully direct spray from wash bottle around tissue, NOT directly on it
10. Immerse in 0.1M Tris 5 min
11. Immerse in 0.1M Tris/2%FBS 5 min
12. Apply 100ul-200ul of Alexa 488 or 594 conjugated linking Ab (Molecular Probes) to section as in step 8 (stock in -80 Histochem use at 1:200 dilution)
13. Incubate at room temp. in humidified chamber for 1hr.
14. Rinse off Link Ab as in step 9
15. Immerse in 0.1M Tris 5 min
16. Immerse in dH₂O 5 min
17. Coverslip with Vectashield with DAPI (Vector Labs)

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, and linking Ab