

DOUBLE LABELING IF 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. Pretreat slides if necessary
5. Immerse in 0.1M Tris buffer (pH7.6) 5min
6. Immerse tissue in 0.1M Tris/2%FBS 30min
7. Prepare primary antibodies together in 0.1M Tris/2%FBS
8. Wipe excess fluid from around tissue; apply 100ul-200ul of Primary Antibody to section
9. Incubate in humidified chamber for 2hrs room temp
10. Rinse off Ab from tissue using 0.1M Tris; carefully direct spray from wash bottle around tissue, **NOT** directly on it
11. Immerse in 0.1M Tris 5min
12. Immerse in 0.1M Tris/2%FBS 5min
13. Apply 100ul-200ul Alexa 488, Alexa 594, or Alexa 647 conjugated linking (secondary) Ab (Invitrogen) to section as in step 8 (stock in -80 Fz use at 1:200 dilution). Apply both secondaries together.
14. Incubate at room temp. in humidified chamber in the **DARK** for 1hr.
15. Rinse off Link Ab as in step 9
16. Immerse in 0.1M Tris 5min
17. Immerse in dH₂O 5min
18. 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.