PATHOLOGY CORE

Double Labeling 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 dH20 1 min

4. Pretreat slides if necessary

5. Immerse in 0.1M Tris buffer (pH7.6) 5 min

6. Immerse tissue in 0.1M Tris/2%FBS 30 min

7. Prepare primary antibodies together in 0.1M Tris/2%FBS

8. Wipe excess fluid from around tissue; apply 100ul-200ul of Primary Antibodies 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 5 min

12. Immerse in 0.1M Tris/2%FBS 5 min

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 1 hr.

15. Rinse off Link Ab as in step 9

16. Immerse in 0.1M Tris 5 min

17. Immerse in dH2O 5 min

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.

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