IMMUNOHISTOCHEMISTRY FOR FROZEN TISSUE

- By Mary Brummet

EXPERIMENTAL PROTOCOL:

1. Bring slides to RT, 10'
2. rehydrate 10, dH2O, RT
3. Place the slides in shanndon slide holders for staining. This is done in a distilled water bath to maintain capaillary action over the tissue. Once in the sequenza rack, wash the slides once with PBS.
4. Dilute the primary antibody and isotype control antibody in the DAKO Antibody Diluent. Add 150ul per slide. Incubate at 37oC, 60'. Wash once with PBS.
5. -IgG Myeloma is diluted 1:1000, 1ug/ml final
6. -Siglec-F/Fc is diluted 1:500, 1ug/ml final
7. Next, apply 150 μL of DAKO Dual Endogenous Enzyme Block to each slide for 15 minutes at room temperature. Wash one time with PBS.
8. Add 150 μL of the biotinylated goat anti human IgG (vector). Incubate for 30 minutes at room temperature. Wash once with PBS.
9. Add 150μl ABC reagent (vector) – streptavidin/alkaline phosphatase. Incubate for 30 minutes at room temperature. Wash once with PBS.
10. Add 150μl Vector Red alkaline phosphatase substrate. Incubate for 20 minutes at room temperature. Wash once with dH2O.
11. Immerse slides in a waterbath to remove from slide holders. Replace slides in 24-count vertical slide holders and dip in hematoxalin dye to counterstain. Immediately rinse slides under running tap water until the water runs clear. Place slides in tap water bath for 5 minutes to equilibrate.
12. Dehydrate the tissues by immersing the slides in a series of ethanol and xylene for 30 seconds each (blotting after each immersion): ethanol 70%, 80%, 95%, 100%, 100%, xylene, and xylene.
13. Leave the slides in the last xylene before coverslipping to prevent the tissue from drying out. Place slides on a cardbaord slide holder and add one drop of Permount (Fisher Scientific) permanent mounting medium and immediately apply

the glass coverslip. Keep the slides in the dark protected from light to prevent the stain from fading.

14. Allow slides to dry at least 24 hours before storing in a plastic slide box.