

## Immunofluorescent Staining on Cryopreserved Sections

\*All steps performed at room temperature in a humidified chamber unless otherwise noted, slides are aspirated between each step\*

1. Fix slides with 4% formaldehyde in PBS [10min]
2. Wash slides in PBS [3x5min]
3. Permeabilize slides in 0.1% Triton X-100 in PBS [10min]
4. Wash slides in PBS [5min]
5. Incubate slides in Blocking Solution [30min]  
\*Blocking Solution: 5% BSA (wt/vol); 0.1% TritonX-100 (vol/vol); diluted in PBS\*
6. Incubate slides in primary antibody in Blocking Solution [1hr]  
\*or incubate overnight at 4°C
7. Wash slides in Blocking Solution [3x5min]
8. Incubate in secondary antibody in Blocking Solution [1hr]
9. Wash slides in PBS [3x5min]
10. Incubate in DAPI in PBS [5min]
11. Wash slides in PBS [3x5min]
12. Remove and Aspirate residual PBS and Mount with Permafluor/Vectashield/Alt.