

IMMUNOHISTOCHEMISTRY - frozen sections
w/ethanol fixation

1. Remove frozen sections from -70 freezer and let thaw
2. RINSE in PBS
3. Immerse slides in 100% EtOH 2 x 5 min. at -20 °C.
4. RINSE - PBS: 5 min. 2x
5. TREATMENT - Hyaluronidase 2mg/ 2ml Sodium Acetate Buffer 5.5, place at 37 for 30 min. in humidity chamber.
6. RINSE - PBS: 5 min. 2x
7. BLOCK in 1% fish gelatin in PBS: 10 min.
*make primary antibody dilutions now
8. PRIMARY ANTIBODY - diluted in Block: overnight 4 degrees Celsius - or 1 hrs. 37°
*cover sections with approximately 200ul - use humidity chamber
9. WASH - PBS: 3x 10 min.
10. SECONDARY ANIBODY - F-GAR diluted 1:1000 - 1:2000 in PBS - 1hour RT
(Note: We use the Alexa 488 & 594 dyes from Molecular Probes, ph: 1-800-438-2209) *keep this solution in the dark and spin it down before use
11. WASH - PBS; 3x 10 min.
12. MOUNT - 90% glycerol in PBS containing approximately 1 mg/ml PPD
OR in Gel/Mount - Aqueous Mounting Media w/anti-fading agents.