

Day 1

1. Mount sections on cleaned Superfrost Plus slides in cold 1x PBS. Remove excess PBS from surface of slide and surround with ImmunoEdge pap pen. Let dry until translucent.
2. Place in hydration chamber, 5-20min 1x PBS rehydration
3. 3x 5min 1x PBS washes using suction method
4. 1hr in permeabilization (perm) solution [**675 μ L BSA stock Q.S. 15mL 0.3% PBT**]
5. 3x 5min 1x PBS washes
6. (OPTIONAL) 20 min Background Buster (Innovex)
7. (OPTIONAL) 3x 5min 1x PBS washes
8. Apply 1^o antibodies diluted in PBS/PBT perm solution (name, company, dilution)
Keep, overnight, at 4°C

Day 2

1. 3x 5min PBS washes
2. Apply 2^o antibodies diluted in perm solution (name, company, dilution)
3. Keep in dark, 1-2hr, at room temp
4. 3x 5min 1x PBS washes
5. Coverslip with ProlongGold (Invitrogen), seal label edge with thick line of nail polish
6. Let dry in dark for 30min-1day
7. Store in dark at 4°C

Notes: Suction method used to remove all liquids from sections. Antibodies are centrifuged before being diluted and vortexed before being applied.

Histochemical grade antibodies should be diluted with PBS that contains either 1.0% BSA or 1% normal goat serum, aliquoted into single use portions, and stored frozen at -20° C: do not repeatedly freeze and thaw. For short term storage, antibody containing solutions can be refrigerated. The addition of 0.01% sodium azide or 0.01% thimerosal to inhibit microbial growth can be used to prolong the length of time antibody containing solutions can be refrigerated: these chemicals should only be used if they will not affect the utilization of the antibodies.