

General Protocol for Immunohistochemistry on Cryosections

① Fix two groups of light txt'd eyes: 4% PFA and 9/1 Eth. Form, O/N 4°C.

- 1X PBS wash 20 min

② Infiltrate for TBS embedding

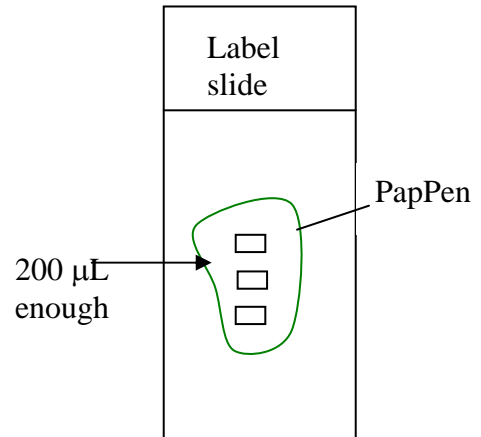
- 5% sucrose/1X PBS 30 min
- 5% sucrose/1X PBS 3 hrs
- 30% sucrose/1X PBS, O/N 4°C
- 30% sucrose/1X PBS:TBS, 1:1, O/N 4°C

③ Embed in TBS

- 100% OCT and freeze

④ Section

- 12-14 μm 3 PER SLIDE
- Dry on warmer (50°C) 2 hrs
- Alt. step: Can freeze at -80°C to store; before continuing, re-dry on warmer for 20 min.
- Outline with PapPen



⑤ Primary antibody incubation

- Hydrate in 1X PBS in coplin jar, 20 min
- Incubate in blocking solution, 1 hr RT
- Dilute primary antibody in blocking soln
- Incubate in primary antibody soln O/N 4°C

<u>Blocking Solution</u>	<u>(10 mL)</u>
2% NGS	200 μl
0.2% Triton X-100	20 μl
1% DMSO	100 μl
in 1X PBS	9680 μl

⑥ Secondary antibody incubation

- Wash 10 min in 1X PBS 0.05% Tween-20 (ex. 50 μl Tw-20 in 100 mL 1XPBS)
- Wash 10 min in 1X PBS 0.05% Tween-20
- Wash 10 min in 1X PBS 0.05% Tween-20
- Dilute secondary antibody in 1X PBS 0.05% Tween-20 (1:500 for AF antibodies)
- Incubate in secondary antibody solution 1 hr RT

⑦ Wash off unbound antibody

- Wash 10 min in 1X PBS 0.05% Tween-20
- Wash 10 min in 1X PBS 0.05% Tween-20
- Wash 10 min in 1X PBS 0.05% Tween-20
- Wash 10 min in 1X PBS

⑧ Coverslip

- Use Prolong Gold or Vectashield