STAINING FROZEN SECTIONS

No fixation – no detergent

1. Add primary antibody in PBS with either 4% BSA or 5% Normal Serum (usually from the species in which the secondary antibody was produced) and incubate for 1-2 hr at room temperature.

2. Wash three times for 10 min in PBS.

3. Add secondary antibody (together with fluorochrome-conjugated α-BGT) in PBS with BSA or Normal Serum and incubate for 1-2 hr at room temperature.

4. Wash three times for 10 min in PBS.

5. Fix for 5 minutes at room temperature with 1% HCHO in PBS.

6. Wash three times for 10 min in PBS.

7. Mount in Vectashield with coverslip.

8. Seal coverslip with nailpolish.

9. Store at 4°C.

With fixation & detergent

1. Fix sections with 1% HCHO in PBS for 5 min.

2. Wash three times for 10 min in PBS.

3. Block sections with either 4% BSA or 5% Normal Serum (usually from the species in which the secondary antibody was produced) for 15 min at room temperature.

4. Add primary antibody in PBS with 0.2% NP-40 and either 4% BSA or 5% Normal Serum (usually from the species in which the secondary antibody was produced) (PBN-B) and incubate for 1-2 hr at room temperature.

5. Wash three times for 10 min in PBN.
6. Add secondary antibody in PBN-B and incubate for 1-2 hr at room temperature.

7. Wash twice for 10 min in PBN and rinse once in PBS.

8. Fix for 5 minutes at room temperature with 1% HCHO in PBS.

9. Wash three times for 10 min in PBS.

10. Mount in Vectashield with coverslip.

11. Seal coverslip with nailpolish.

12. Store at 4°C.

**FIXATION:**
1. Prepare a solution of 1% formaldehyde in 0.1 M NaPi, pH 7.3 (for 25 ml): add 0.25 g of paraformaldehyde to ~10 ml of H₂O. Warm to ~60°C on a heating plate, in a fume hood, and add 1 N NaOH, dropwise, swirl, until the paraformaldehyde has dissolved. Cool to room temperature. Add 12.5 of 0.2 M NaPi, pH 7.3, and top-off with H₂O to 25 ml. Check pH with pH paper – never with a pH meter.

**SYNAPSES**
1. Collect sections from a region of muscle that is known to be enriched in synapses. Confirm that synapses are present in sections, as you collect them, by staining a single section, every several hundred µm, for AChE. The stain appears in a minute or two, and is best visualized with a 25-40X water immersion lens.