**Immunofluorescence protocol for frozen sections**

**Procedure: Fixation**

1. Collect tissue in 4% PFA and incubate at 4o C for overnight.

2. Wash with PBS, transfer tissue to 30% sucrose in a conical tube or screw-cap vial and store at 4o C overnight.

**Procedure: Preparing Frozen Sections**

1. Remove tissue from sucrose and drain excess solution. Place the tissue sample in the OCT compound, keeping track of tissue orientation. Adjust the orientation with a probe or closed watchmaker forceps if necessary.

2. Add OCT compound if necessary to fully cover the tissue sample.

3. Snap freeze the block in dry ice, Frozen blocks can be stored at -80o C for several days, or immediately sectioned.

4. Mount a disposable blade and place all necessary items in the cryostat: 3-4 kimwipes, brush, etc. Set the cryostat to approximately -22o C and allow temperature to equilibrate. Set the section thickness, typically 10 µm for sciatic nerve.

5. Label the slides numerically with pencil

6. Apply a small base of OCT compound to a sectioning pedestal inside the cryostat. Mount the frozen sample block in this base and press “Freeze Object.”

7. Make sure that the sectioning arm is moved sufficiently back from the blade that the pedestal and block can be mounted without touching the blade. Mount the pedestal with block in the holder, with the desired orientation.

8. Slowly raise the sectioning arm nearly to the blade. Proceed further advancing the arm as though sectioning, until the blade begins to cut into the OCT block.

9. Wipe away unwanted or damaged sections with a brush or kimwipe, always moving from the back of the blade to the front. Allow the blade a few moments to return to the proper temperature before resuming sectioning.

10. Sections may be collected singly or in a short string of 2 – 4 by holding a pre-warmed slide just above the sections on the blade. Check sections under a dissecting microscope and keep notes about section location and quality on specific numbered slides for later reference. Return slides to the room temperature to dry.

11. Always clean the blade with brush or kimwipe after collecting sections and allow temperature to reequilibrate.

12. Slides should be may be stored in a slide box at -20oC/-80oC for weeks to months before immunostaining.

Tips and tricks:

• If sectioned material begins to shrink immediately, cryostat temperature is too warm.

• If sections begin to curl, cryostat temperature is too cold.

• Distortion of sections may also result from excess OCT compound surrounding the tissue.

**Procedure: Immunostaining Frozen Sections**

Do not let slides dry out after starting the procedure.

1. Warm slides to room temperature for approximately 30 minutes.

2. Cover sections with ~200µl blocking solution (5-10% Goat/Donkey serum), place in the humidity box and incubate for 30 minutes-one hours at room temperature.

3. Gently flick away the blocking solution and add primary antibodies diluted in blocking solution or PBST. Incubate in humidity box at room temperature for 30 minutes to several hours, or overnight at 4oC.

4. Wash slides three times with PBS in a staining jar at room temperature for 10 minutes per wash.

5. Wick away excess PBS with a lab wipe.

6. Add secondary antibody diluted in blocking solution or PBST. Incubate at room temperature for 30 minutes to one hour in humidity box.

7. Counter stain with DAPI or nuclear stain of choice diluted in PBST.

8. Wash twice for ten minutes in PBST at room temperature.

9. Mount slides with mounting medium and a no. 1.5 cover glass.