Immuno Fluorescence Method for Semithin Cryosections:

Usually carried out in a coplin jar, with antibody incubations in a special incubation chamber.

1. Make sure that the (groups of) sections are separated on the microscope slides with a wax pen, so that no cross-contamination with different antibodies can occur.

2. Place slides in coplin jar and rinse thoroughly with PBS to get rid of any sucrose / methyl cellulose. Exchange the PBS multiple times and agitate gently.

3. Wash once with 0.15% glycine in PBS for 10 minutes.

4. Block with 5 to 10% serum (i.e. fetal calf serum or normal goat serum) in 1% BSA / PBS for a minimum of 20 minutes. Some cold water fish skin gelatin can be added too (~1%).

5. Take slides out of coplin jar (one at a time to prevent drying of the sections), place them in an incubation chamber, carefully remove the blocking solution and replace it with the primary antibody diluted in 1% BSA / PBS. Make sure the sections are covered (usually takes about 25 µL per group of sections) and keep the camber humid with moist tissue. Leave for 1 to 2 hours at room temperature.

6. Wash slides 3 times (~ 5 min each) with 0.1% BSA / PBS in the coplin jar.

7. Prepare secondary antibody dilutions in 1% BSA / PBS, and apply as before. Leave for 45 minutes to 1 hour. Most of our Alexa conjugated secondaries are diluted 1/500. Include DAPI in this step, as this will make it much easier to find the sections.

8. Wash with PBS (without BSA) 3 times in the coplin jar.

9. For each microscope slide, pipet about 3 µL of AFR onto each area with sections and very carefully place a large coverslip onto the AFR droplets. Avoid trapping bubbles.

10. Nail polish can be used on each corner, but make absolutely sure that it has dried completely before using a microscope, so you don’t contaminate any objectives.

AFR = Anti Fading Reagent = 1% n-propyl gallate (Sigma P-3130) in glycerol/PBS 1:1
Gently heat the glycerol/PBS 1:1 mixture, add n-propyl gallate and vortex until dissolved. Store in freezer.