Immunofluoresence on Semi- Thin Sections

A). Fix tissue, or cells with paraformaldehyde, or PLP fixative. (Gluteraldehyde
fixed tissue or cells may auto fluoresce.)
B). Trim cell pellet, tissue, in fixative into small pieces (<=1mm sq.)
C). Infuse with PVP (20g Polyvinylpyrrolidone (MW 10,000) in 100 ml 2.3M sucrose), or 2.3M sucrose for at least one hour. (Two hours is optimum duration, However I have not seen serious morphological compromise when tissue was exposed to PVP-sucrose overnight at four degrees.
D). Freeze specimen in liquid nitrogen.
E). Cut semi-thin sections (0.5-2.0um) and place them in a cluster in the center of a coated slide. Prescore the slide with a diamond scribe.

1. Place slides in a humid chamber, cover generously, but gently with plain PBS. Wash away the sucrose, wash for 15-20 minutes. With multiple changes of washing buffer. Sucrose has a high affinity for fluoresence molecules.
2. Incubate sections with a blocking buffer, to quench nonspecific binding. 1X PBS, 10% FCS, with Azide 0.1%. Up to one hour.
3. Wash off excess blocking buffer, make wash buffer, (1X PBS + 0.05% Glycine, 0.75 % BSA)
4. Dilute primary antibodies in 10% FCS, PBS buffer with 0.1% azide. Incubate antibodies for at least one hour.
5. Wash sections gently, 5-6 washes 5-10 mins each.
6. Wipe off excess buffer, don't let sections dry out, add secondary antibody, incubate for at least one hour.
7. Wash sections multiple times as in step 5. Cover slip using a anti- bleaching agent such as PPD. Seal coverslips with fingernail polish.

Recipe for PPD

10mg p-phenylene diamine
in 1.5 ml DI H_2O
and 1ml 10X PBS,
Sonicate this solution breifly, to dissolve the PPD.
add glycerol upto 10 ml.
Store coverd in foil at -20

Protocol taken from the W.M. Keck Microscopy Facility at The Whitehead Institute website