Immunofluorescence

of Frozen Tissue Sections

Supplies and Reagents

- Acetone
  - stored at -20°C
- 20X PBS
  - 43.6g Na_2HPO_4 (sodium phosphate dibasic anhydrous)
  - 12.8g NaH_2PO_4 (sodium phosphate monobasic anhydrous)
  - 360.0g NaCl (sodium chloride)
  - bring up to 2L with H_2O
  - pH to 7.2
- 1X PBS
  - dilute 20X PBS with H_2O
- Blocking Buffer in PBS (use following recipe or alternatively use 10% BSA in PBS)
  - 10% normal serum (host species of secondary antibody)
    - Horse Serum (Jackson ImmunoResearch 008-000-121)
    - Goat Serum (Jackson ImmunoResearch 005-000-121)
  - 1% BSA
- Primary Antibody
  - antibody against the protein of interest (γ-H2A.X, active caspase-3, CD3, etc.) and raised in an animal other than mouse
  - diluted at an appropriate concentration in blocking buffer
- Secondary Antibody
  - antibody against the host species of the primary antibody
  - conjugated to a fluorescent dye (Fluorescein, Texas Red, etc.)
  - diluted at an appropriate concentration in 1X PBS
- DAPI (Sigma D9564-10MG)
  - dilute in PBS
  - store at 1mg/mL
  - use at 0.5μg/mL
- Superfrost/Plus Microscope Slides (Fisher 12-550-15)
- Slide Rack
  - will hold up to 24 slides
- Wash Container
  - slide holder will fit into the container
- Cover Slips 22x30-1.5 (Fisher 12-544-D)
- ImmEdge Pen (Vector Laboratories H-4000)
- Mounting Medium (Sigma G0918-20ML)
- Humidified Chamber
  - can be made with any sealing plastic container and damp paper towels
- Clear Nail Polish (with applicator)

***Fresh-frozen tissue sections, embedded in OCT, should have already been cut onto Superfrost/Plus slides at a thickness of 10μm**

***Slides should have been stored at -20°C until ready to use**

***All slide washes take place using the slide rack and wash container in 1X PBS**

***A negative control is needed for each run to test the secondary antibodies reaction to the tissue, or background (use protocol as normal, but for one section, replace primary antibody with blocking buffer)**

I. Fix and Permeabilize

1. Remove slides from freezer, insert into slide rack, place into wash container with -20°C acetone, and fix/permeabilize for 10 minutes.
2. Remove rack, set slides on paper towel, and allow to air dry.
3. Using the ImmEdge pen, draw a hydrophobic barrier around each tissue section. **Note: to conserve reagents, draw barrier close to, but not on, sections.**
4. Wash slides three times, 5 minutes each, in PBS.

***Do not allow tissue sections to dry past this point**

II. Block

1. Remove slides from rack, tap off excess PBS onto paper towel, and lay flat in humidified chamber.
2. Add enough blocking buffer to cover each tissue section, using a pipet. **Note: amount of buffers/antibody/DAPI needed will depend on the section size, but is typically less than 100μL per section.**
3. Close off humidified chamber and incubate for 1 hour at room temperature.
4. Wash slides three times, 5 minutes each, in PBS.

III. Primary Antibody

1. Tap off excess PBS onto paper towel and lay flat in humidified chamber.
2. Add primary antibody to each section.
3. Close the humidified chamber and incubate overnight at 4°C.

IV. Secondary Antibody

1. Wash slides three times, 5 minutes each, in PBS.
2. Tap off excess PBS onto paper towel and lay flat in humidified chamber.
3. Add secondary antibody to each section.
4. Close humidified chamber, protect it from light, and incubate for 1 hour at room temperature.
5. Wash slides three times, 5 minutes each, in PBS.

V. DAPI

1. Tap off excess PBS onto paper towel and lay flat on paper towel.
2. Add DAPI to each section.
3. Protect from light and incubate for 10 minutes at room temperature.
4. Wash slides two times, 5 minutes each, in PBS.

VI. Mount and Cover Slip

1. One slide at a time, remove from PBS and tap off excess onto paper towel.
2. Lay flat on a paper towel and remove any remaining PBS outside of barriers with a folded Kim wipe.
3. Add 3 drops of mounting medium to the slide.
4. While trying to exclude bubbles, lower a cover slip to cover all sections, using a razor blade to position it.
5. Allow to dry for 1 hour at room temperature in the dark.
6. Seal edges of cover slip with nail polish, and allow 15 minutes to dry in the dark.
7. Store slides at 4°C in the dark.