

## Immunofluorescence on cultured cells

1. Fix cells for 1 hour in 2% Paraformaldehyde in .1M phosphate buffer. (pH 7.4) At room temperature. Cells can be held at this point in 0.5% Paraformaldehyde.
2. Rinse cells with multiple changes of PBS 30 minutes. pH 7.4
3. Permeabilize cells with either,
  - (a) 0.1% Triton-X100 in PBS for 10 minutes.
  - (b) 0.05% Saponin in PBS/0.1% BSA, 0.075% glycine, for 10 minutes.

*If using Triton-X100 follow (a)*

### **a**

1. Wash out the Triton-X100 using PBS, wash multiple times (5-6)
2. Rinse for 15 minutes with blocking buffer. (1X PBS, 0.1% BSA, 0.075% Glycine) Do not let cells dry out between washing
3. Dilute primary antibodies in blocking buffer. Incubate cells with the primary antibody for one hour in a humid chamber.
4. Wash cells multiple times with the blocking buffer for 30 minutes.
5. Dilute secondary antibodies in the blocking buffer. Incubate cells with the secondary antibodies for one hour in a humid chamber.
6. Wash cells multiple times with the blocking buffer. Mount coverslips onto glass slide using a mounting medium with an anti-bleaching agent.

*If using saponin follow (b)*

### **b**

1. After cells have permeabilized, Wash once with the Saponin buffer.
2. Dilute primary antibodies in Saponin buffer. Incubate cells with the primary antibodies for one hour in a humid chamber.
3. Wash cells multiple times with Saponin buffer for 30 minutes.
4. Dilute secondary antibodies in Saponin buffer. Incubate cells with secondary antibody for one hour in a humid chamber.
5. Wash cells multiple times with Saponin buffer for 30 minutes.
6. Wash cells 2 times with plain PBS for 10 minutes.
7. Mount coverslips onto glass slide using a mounting medium with an anti-bleaching agent.

Coverslip can be sealed with finger nail polish, and slides can be stored at -20.

## **Mounting Medium. PPD**

10mg P-1Phenylene diamine  
1.5ml DI H<sub>2</sub>O  
1ml PBS (10X)

Sonicate the above solution to dissolve the PPD.  
Add Glycerol upto 10ml, mix thoroughly.  
Store covered in foil at -20.

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