

Immunofluorescence Protocol

1. Fix in 4% paraformaldehyde
2. Permeabilize in 0.2% triton x-100
3. Rinse in 1 x PBS
4. Block for 1 hour in 10% goat serum, 1% BSA in PBS.
5. Rinse with PBS
6. Dilute primary in PBS in 1% BSA. Try 1:200 or something like that.
7. Incubate an hour or so, or overnight at 4C
8. Rinse 3x
9. Incubate in secondary in 1% BSA in PBS.
10. Rinse 3x. At this point you can do the dapi or f-actin phalloidin staining too.

Protect from light whenever handling the secondary antibody, as well as your samples after you've added the secondary.

Just add the antibodies directly on the transwell surface. 100 ul is enough to cover the surface. When rinsing use a high volume of PBS.