Sugden Lab.

Immunofluorescent Staining

Note:

- For suspension cells, keep cell concentration less than $1x10^7$/ml.
- Adhersion cells grown on a 22x22mm coverslip can be stained with 150 ul of antibody solution. I usually drop the antibody solution on a piece of parafilm, and put the coverslip onto the drop.
- Be generous with time and buffer spent on blocking and washing.

1. Fix cells with 4% formaldehyde for 15-30 min.

2. Permeablize cells with 0.05% Triton X-100 at room temperature for at least 10 min.

3. Rinse cells with PBS twice

4. Block cells with 2-5% calf serum in PBS for 30min at room temperature.

5. Stain with primary antibody diluted in 5% CS+PBS

6. Wash with 5% CS+PBS two times

7. Stain with secondary antibody diluted in 5% CS+PBS

8. Wash with 5% CS+PBS three times. (I usually do 5min for each washing)

9. Mount cells on slides with 50% glycerol. For long term storage, you may want to mount cells with solution containing anti-fade reagents (Vector lab sells it).