

Staining of Cultured Cells

Cells can either be stained live and fixed after adding secondary antibody or they can be fixed before staining .

Staining fixed cells:

1. Remove medium from cells and wash 2-3 times with PBS.
2. Fix cells with ice cold 4% paraformaldehyde for 10 minutes.
3. Wash 3X with PBS.
4. Add primary antibody for 30 minutes - 1 hr at RT.
5. Wash 3X with PBS.
6. Add secondary antibody for 30 minutes at RT. We usually use FITC or Cy3 conjugated antibody (Jackson 1/500- 1/1000).
7. Was 3X with PBS.
8. Coverslip with Vectashield and observe.

Staining live cells:

1. Same as above except that you stain with primary and secondary antibodies and then fix with 4% PFA for 10 minutes.
2. Wash 3X with PBS.
3. Coverslip with Vectashield and observe.