Immunofluorescence protocol

Cells.
Cells grown on cover slips or on commercially available incubation chambers.

Solutions / Reagents:
1. PBS and cPBS (complete PBS)
2. Fixative 4% formaldehyde in PBS (freshly prepared)
3. Quenching solution: 50 mM NH4Cl in PBS or 0.1M Glycine in PBS
4. Blocking solution 1% BSA or 10% FCS (fetal calf serum) in PBS
5. Blocking and permeabilization solution 10% FCS + 0.1 Triton X100 or 0.1% Tween100
6. 1st antibody diluted in the blocking solution + 5% serum of the animal host of the secondary antibody (goat, donkey, sheep, etc)
7. 2^{nd} antibody diluted in the blocking solution
8. Mounting media with an antifading reagent (Fluoromount, Slow Fade, Vectashield works well, except for cy2).

Protocol
- Day 1.
  Place the sterile cover slips in 12 or 24 well plates; rinse the cover slips with PBS, followed by a quick rinse with culture media.
  Plate the cells on the cover slips at a density of ~ 10,000/ cm^2
- Day 2

1. Rinse the cells with cPBS
2. Fix the cells with freshly made fixative, for 30 min
3. Wash gently with PBS, for 2 min, twice
4. Quench with 50 mM NH4Cl for 15 min
5. Wash with PBS, for 5 min
6. Block /and permeabilize (if the 1^{st} antibody is against a cytoplasmically domain of the protein or is present intracellularly) for 1 hr., at RT.
7. Incubate with the 1^{st} antibody in the blocking/permeabilization solution at RT for 1 hr, or ON at 4°C.
8. Wash with PBS- three times for 5 min with gentle shaking
9. Incubate with 2^{nd} antibody for 1 hr (light protected)
10. Wash for three times for 5 min with PBS
11. Pipette 20-30 µl of antifading agent /Vectashield on clean glass slides
12. Rinse the cover slip with distilled water and mount the cover slips with the cells facing down on the glass slides. Remove excess liquid with filter paper. Seal the cover slips with polish nail.

Controls
1. Omit the first antibody(ies), incubate the samples with secondary antibody(ies) to check for unspecific binding or for cross-reaction between the secondary antibodies.

2. Incubate the samples with the pre-immune serum (if it is available, or ‘normal” rabbit, rat, mouse IgG) instead of the first antibody, followed by secondary antibody(ies) to check for nonspecific binding.

Keep the samples light protected in the refrigerator or in the freezer.
Scope the samples in the next 48-72 hrs. The fluorescence is usually preserved for weeks if the antifading agent is good.