Immuno-Fluorescence standard protocol:

1. Grow cells on cover slips -> treatment
2. Keep cells on ice
3. Wash once with cold PBS (replace with TBS for phospho-proteins)
4. Fix cells for 30 min in 4% paraformaldehyde @4°C. **Note 1!**
5. Quench fixation with 50 mM NH_4Cl in PBS for 5 min at RT. (Cell may be kept @4°C for a few days in PBS after this step)
6. Permeabilize cells with 0.2% Triton X-100 in PBS at RT for 15-30 min (cell type dependent)
7. Wash once with PBS
8. Block with 1-5% BSA in PBS (primary ab dependent) for 30-45 min at RT (0.05-0.1% Triton X-100 may be added to buffer to improve signal/noise)
9. Wash once with PBS
10. Apply Primary antibody in 1-5% BSA overnight @4°C (or 45-60 min @ RT). Adding 0.05-0.1% Triton X-100 may improve low specificity. New ab need to be optimized for concentration.
11. Wash quickly twice and once for 20 min with PBS. Ab with high sensitivity and low specificity may need more extensive washing.
12. Apply secondary antibody (1:500-1000 in 1% BSA) in PBS for 45-60 min. NB! Beware of light conditions
13. Wash once with PBS
14. Stain with DAPI in PBS for 10 min @ RT
15. Wash 2 x 5 min with PBS
16. Wash once in sdH_2O (desalting)
17. Mount cover slides with Mowiol. Let dry @ RT overnight (If in hurry: dry at 37 degrees for 30 min)
18. (If needed: seal slides with nail polish)
19. Keep slides in dark at @4°C

General tips:

Make sure PBS has pH 7,4-7,6.
After applying primary antibody make sure to work with the samples away from light.
Don't let cells dry.
As standard: use AF488+AF555+DAPI combination

Note 1: Some ab works better with methanol fixation. This replaces step 4-7:

4. Aspirate liquid, then cover cells to a depth of 2–3 mm with ice-cold 100% methanol
5. Allow cells to fix for 5-15 minutes at -20°C (cell dependent)
6. Wash 3 x 5 min with PBS
7. Proceed with step 8