

Protocol for ICC

1. **Fixation** : Aspirate liquid, wash cells seeded on clean coverslips with 1X PBS three times for 3min. Then fix the cells with fresh 4% formaldehyde in PBS for 15 min at room temperature.
2. **Rinse 3X in PBS**: Aspirate fixative, rinse three times in PBS for 5 min each.
3. **Permeabilization**: Permeabilize with 0.1% Triton X-100 made in 1X PBS for 20 minutes.
4. **Rinse 3X in PBS**: Aspirate fixative, rinse three times in PBS for 5 min each.
5. **Blocking**: Remove the residual, add 5% BSA or normal serum on the slides. Make sure the serum is from the same species of second antibody and not immune. Then incubate for 20min at room temperature.
6. **Incubate primary antibody**: Remove the residual, apply diluted primary antibody at a appropriate dilution for each coverslip, then incubate overnight at 4°C.
7. **Rewarming of slide**: Move the slide from 4°C to room temperature for about 20min to avoid the off-slide for rinse immediately.
8. **Rinse 3X in PBST**: Remove the residual, soak in PBST, and rinse in shaker at 40 rpm, 5min each time.
9. **Incubate with fluorochrome-conjugated secondary antibody**: Remove the residual, apply diluted fluorochrome-conjugated secondary antibody at a appropriate dilution for each coverslip, then incubate 60min at 4°C in the dark.
10. **Rinse 3X in PBST**: Remove the residual, soak in PBST, and rinse in shaker at 40 RPM, 5min each time.
11. Add 50% PBS and 50% glycerol to the slide and then observe specimens under a microscope immediately.

NOTE:

1. Formaldehyde is toxic, use only in fume hood.
2. The primary antibody concentration, incubation time and temperature directly affect the staining efficiency and background staining. If the positive staining is too weak, the concentration of the primary antibody and the incubation time can be increased; if the background is too high, the primary antibody concentration and the incubation time can be decreased.
3. As fluorescence can photobleach when exposed to light, so incubating with secondary antibody and the rinse process should be done in the dark.