Immunofluorescence Guide

Fixation and permeabilization

1. Wash the cover glass seeded with cells in the culture plate with 1× PBS buffer for 3 minutes and repeat 3 times.
2. Fix the cultured cells for 15 minutes with 4% paraformaldehyde, and then wash the cover glass with 1× PBS for 3 minutes and repeat 3 times.
3. Permeate the cells at room temperature for 15 minutes with 0.5%Triton X-100 (prepared with 1× PBS) (This step can be omitted for antigens that are expressed on cell membranes), and then wash the cover glass with 1× PBS for 3 minutes and repeat 3 times.)

Blocking

4. Blot up the absorbent paper with 1×PBS, and add 5% normal serum (Sharing the same or similar species with secondary antibodies) drop by drop on the cover glass, then incubate it at room temperature for 1 hour.

Antibody incubation

5. Use absorbent paper to aspirate the blocking solution without washing the cover glass, and add sufficient and diluted primary antibodies drop by drop on each cover glass and then put them into wet box to be incubated at 4℃ overnight.
6. Add fluorophore-conjugated secondary antibodies: firstly wash the cover glass with PBST for 3 minutes and repeat 3 times, and then add the diluted fluorophore-conjugated secondary antibodies drop by drop after blotting up the redundant liquid on the cover glass with absorbent paper. Finally put them into wet box to be incubated at 37℃ for 1 hour, and wash the section with PBST for 3 minutes and repeat 3 times. Attention: All of the operation steps should be operated in the dark after adding the fluorophore-conjugated secondary antibodies.

Fixing and taking pictures

7. Nuclear staining: add DAPI drop by drop and incubate for 5 minutes in the dark, and then stain the nucleus of the specimen. Finally wash away the redundant DAPI with PBST for 5 minutes and repeat 4 times.
8. Blot up the liquid on the cover glass with absorbent paper, then use antifade mountant to mount the cover glass, finally observe and collect images under a fluorescence microscope.