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General Immunofluorescence Staining Protocol using Indirectly Conjugated Antibodies

1. Test principle

Cells are stained using indirectly conjugated antibody for immunofluorescence

2. Specimen

Cells in suspension, from whole blood, bone marrow or cell culture

3. Materials and reagents

- 5ml Polypropylene test tubes (12x75 mm, round bottom)
- cooling centrifuge
- sterile-staining buffer (PBS, 2% FCS, 0.1 % azide) : The sodium azide assists in preventing capping and shedding or internalization of the antibody-antigen complex after the antibodies bind to the receptors.
- primary monoclonal antibody
- secondary antibody conjugated with fluorochrome
- Fc Blocker
- Ice
- 1% parafomaldehyde in PBS
- flow cytometer

4. Controls

- Unstained cells
- Single stained controls in multicolor assays
- Cells stained with secondary antibody only
- Isotypic IgG controls
- FMO control (= fluorescence minus one) in multicolor assays (Optional): Incubate with all colors except the one, you are interested in for that particular tube $\Rightarrow$ negative control for color of interest

5. Procedure

1. Prepare single cell suspension and wash in staining buffer (PBS, 2% FCS, 0.1% azide).
2. Centrifuge (300 x g, 5 min, 4°C.), discard supernatant and resuspend to 1 x 10^7 cells/ml with staining buffer.

3. Aliquot 100 ul of cells (10^6 cells) into a 12 x 75 mm polypropylene FACS tube.
4. Add 5 µl / tube of blocking antibody or serum (e.g. Fc Block).
5. Vortex and incubate for 2 min at room temp.
6. Add primary antibody, vortex gently and incubate for 30 min at 4°C (on ice) in the dark.
7. Add 2 ml of staining buffer, vortex gently and centrifuge (300 x g, 5 min, 4°C).
8. Discard supernatant, resuspend cells in 100 µl staining buffer and add 20 µl secondary antibody.
9. Vortex gently and incubate for 30 min at 4°C on ice in the dark.
10. Add 2 ml staining buffer, vortex and centrifuge as previous.
11. Discard supernatant.
12. Wash again in 1 ml staining buffer and resuspend in 500 µl staining buffer for flow cytometry analysis.
13. Keep cells on ice prior to analysis.

14. Cells may be centrifuged and fixed in 1 ml of 1% paraformaldehyde (in PBS) at 4°C for analysis next day.

Note:

- You might need to adjust cell numbers, amount of antibody for your experiment.
- Use buffers without Phenol Red.
- The blocking antibody step (4 and 5) is optional but should be included if cells express high levels of Fc receptors which will contribute to non-specific binding and background fluorescence.