Direct Staining Protocol	Date: 04-08-2016	page 1 of 1

General Immunofluorescence Staining Protocol using Directly Conjugated Antibodies

1. Test principle

Cells are stained using directly 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.
- monoclonal antibody conjugated with fluorochrome
- Fc Blocker
- 1% parafomaldehyde in PBS
- flow cytometer

4. Controls

- Unstained cells
- Single stained controls in multicolor assays
- 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 (400 x g, 5 min, 4°C.), discard supernatant and resuspend by first flicking bottom of tube to break up pellet, and add staining buffer to 1 x 10^7 cells/ml.
3. Aliquot 100 µl of cells (10^6 cells) into a 12 x 75 mm polypropylene tube.
4. **Blocking:** Add 5 µl/tube of blocking antibody or serum (e.g. Fc Block). Pipetting up and down to mix and incubate for 2 min at room temp.
5. **Surface antibody staining:** Add monoclonal antibodies to the tube (turn off hood light for antibodies), pipetting up and down to mix and incubate for 30 min at 4°C **in the dark**.
6. Add 2 ml of staining buffer, mix well and centrifuge (400 x g, 5 min, 4°C).
7. Discard supernatant.
8. Wash again in 1 ml staining buffer and resuspend in 500 µl staining buffer for FACS analysis.
9. Keep cells on ice prior to analysis.
10. Cells may be centrifuged and fixed in 1 ml of 1% paraformaldehyde (in PBS) at 4°C for analysis next day.

Note:

- 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.
- You might need to adjust cell numbers, amount of antibody for your experiment.