

## Intracellular Staining Protocol

1. Add 50  $\mu$ l of cell suspension to be analysed (up to  $10^6$  cells) into a 12x75 mm cytometer tube.
2. For each sample, add an appropriate volume of conjugated antibody directed to the cell surface antigen of interest and the appropriate isotype control. Incubate for 15 minutes in the dark at room temperature (20-25°C) or for 30 minutes at 4°C. (*This step is only necessary if you want to perform a direct immunofluorescence staining for a cell surface antigen*).
3. Add the suggested volume of Fixative Reagent to each tube. Mix gently.
4. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 minutes at 4°C.
5. Add 2 mL of wash solution and resuspend the cells. Mix well
6. Centrifuge for 5 minutes at 300xg and carefully aspirate the supernatant so as not to touch the cell pellet. Leave 50  $\mu$ l of non-aspirated liquid.
7. Add the appropriate volume of Permeabilization reagent, to each tube. Add the appropriate volume of conjugated intracellular antibody specific for the intracellular antigen and the appropriate isotype control.
8. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 minutes at 4°C.
9. Add 2 mL of wash solution and resuspend the cells. Mix well.
10. Centrifuge for 5 minutes at 300xg and carefully aspirate the supernatant so as not to touch the cell pellet.
11. Resuspend the pellet in 0.3 ml of flow cytometry solution.

Acquire on a flow cytometer or store in the dark at 2°-8°C until the analysis is carried out.

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. ([tech@immunostep.com](mailto:tech@immunostep.com))

### Reagent list:

- Fixative reagent: Intracell (Ref.: INTRACELL-IOOT) Reagent A
- Permeabilization reagent: Intracell (Ref.: INTRACELL-IOOT) Reagent B
- Flow cytometry solution: 20 Mm  $\text{NaH}_2\text{PO}_4$ , 150 NaCl, pH 7.2 + 1% Paraformaldehyde.
- Wash solution: 20 Mm  $\text{NaH}_2\text{PO}_4$ , 150 NaCl, pH 7.2 + 0,09% Sodium azide ( $\text{NaN}_3$ ) + 0,5 % bovine serum albumin.
- Isotype control: <http://immunostep.com/22-isotype-controls>