# FACS Protocol: Surface and Intracellular Staining of Human Whole Blood

## A. Surface staining

- Add **100 µl** of whole blood in 5ml BD falcon (FACS tube).
- Add **10 µl** of each antibody (commercial) in every sample. Add appropriate isotypic controls.
- Mix gently and incubate in a dark place for *15 min* at room temperature.
- Add 2ml **BD lysing solution 1x** in every sample.
- Vortex for *10 sec.*
- Incubate in a dark place for *15 min* at room temperature.
- Centrifuge at 1000 rpm for *5 min.*
- Discard supernatants.
- Add 1ml **PBS 1x** without Ca or Mg.

## B. Intracellular staining

- For each FACS sample use 0.5 - 2 x $10^6$ cells/tube.
  Wash cells by adding 3 ml **5% PBS/FCS** per sample.
  Centrifuge at 200 x g for *5 min.*
- Aspiration (100-150 µl) - Discard supernatants.
- Fix by adding **100 µl** Reagent A (monoclonal-mouse)
  Mix gently by hand and incubate for *15 min* at room temperature.
- Wash cells by adding 3 ml **5% PBS/FCS.**
  Centrifuge at 200 x g for *5 min*.
- Aspiration – Discard supernatant leaving some covering the cells.
- Permeabilization – Add **100 µl** Reagent B and mix gently.
- Add **1.8 µl** of Antibody for TF.
  Spin
  Incubate for *3 h* at 4 °C.
- Wash cells by adding 3 ml **PBS/FCS** and mix gently.
  Centrifuge at 200 x g for *5 min*.
- Aspiration – Discard supernatants.

- Add 10 µl secondary Antibody (anti-mouse) and incubate for *30 min* at 4°C.
  Vortex.
  Wash cells by adding 3ml **5% PBS/FCS**.
  Centrifuge at 200 x g for *5 min.*