Intracellular Staining Protocol

1) Fixation and permeabilization that are required for detection of intracellular antigens may alter the light scatter properties of cells and may increase non-specific background staining. Increasing concentration of BSA/FBS/BCS in the FACS buffer may help reduce the non-specific background. It is **highly recommended** to use of dead-live dyes to aid in the analysis.

2) Add 1-3 × 10^6 cells in a tube (1.5 ml). To eliminate potential artifacts due to dead cell contamination, use a dead-live marker (See Viability Protocols) for the exclusion of dead cells from the analysis.

3) Stain cells surface antigens (Refer to General Surface Staining Protocol). After last wash, discard supernatant.

4) After last wash, vortex to dissociate the pellet. Fix cells by adding 500 µL of 4% paraformaldehyde (PFA) soln. while vortexing the tube. Incubate 15-30 min/dark/RT (or 4°C).

5) Wash 1X with 1X PBS/1% FBS* (or BCS*). Centrifuge at 300-400xg/4°C. Discard supernatant. Wash 1X with Permeabilization Buffer. Centrifuge, discard supernatant.

6) Resuspend cells in 100 µL of Permeabilization Buffer. Incubate 10-15 min/dark/RT (or 4°C). Add the recommended amount of fluorochrome-labeled antibody for detection of intracellular antigens. Incubate 30 min/dark/RT (or 4°C).

**NOTE:** Ab must to be in Permeabilization Buffer!!

7) Wash 2X with FACS buffer. Centrifuge sample 300-400xg/5 min, discard supernatant. Resuspend pellet in an appropriate volume of FACS buffer (you may also fix cells with 4% PFA), acquire data on flow cytometer.

*FBS=Fetal Bovine Serum; BCS=Bovine Calf Serum

**Permeabilization Buffer**

Dulbecco’s PBS (without Ca^{2+} or Mg^{2+})

1% heat-inactivated FCS

0.1% (w/v) sodium azide

0.1% (w/v) saponin (i.e. Sigma, Cat # S-7900)

Adjust buffer pH to 7.4-7.6. Filter sterilized.

For those just starting out, we recommend the use of Fix/Perm kits (i.e. BD Biosciences Cat#: 641776; Affymetrix/eBiosciences Cat #: 88-8824-00; LifeTechnologies Cat #: GAS003; MACS Miltenyi Biotec Cat #: 130-090-477; etc.).