ROCKLAND

Flow Cytometry (FC) Protocol

Flow cytometry combines cell biology with the study of light waves and employs instrumentation that scans single cells flowing past excitation sources in a liquid medium. The technology can provide rapid, quantitative, multi-parameter analyses on single living (or dead) cells based on the measurement of visible and fluorescent light emission. Flow cytometry is a widely used method for characterizing properties of biological cells, including size, granularity, cell receptors, and even biological activities, such as DNA and RNA replication and protein synthesis or suppression.

This basic protocol focuses on the measurement of fluorescence intensity produced by fluorescent-labeled antibodies and ligands that bind specific cell-associated molecules.

I. Materials

Product
Fast FACS Buffer (MB-086-0500) **Note:** Sodium azide assists in preventing capping and shedding or internalization of the antibody-antigen complex after the antibodies bind to the receptors. We suggest using our 10X PBS (#MB-008) (10X PBS pH 7.2 (0.2 M Potassium Phosphate, 1.5 MSodium Chloride).
Fixation Buffer: 2% Paraformaldehyde in PBS
Permeabilization Buffer: 0.1% Triton in PBS
70% Ethanol, stored at -20°C

II. Procedure for Cell Surface Antigens Analysis

1. Collect 1–3 x 10^5 cells per sample. Wash with 2 mL FACS buffer.
2. Centrifuge and aspirate supernatant.
3. Resuspend cells with 100 µL FACS buffer containing 0.5–1 µg antibody. Vortex and incubate on ice for 30 minutes.
4. Wash cells with 2 mL FACS buffer.
5. Centrifuge and aspirate supernatant.
6. Resuspend cells with 100 µL FACS buffer containing 1 µg secondary antibody. Vortex and incubate on ice for 30 minutes.
   Note: Perform the incubation in the dark. Secondary antibodies used for FACS are typically F(ab')2 fragment antibodies conjugated to fluorochromes like FITC or R-Phycoerythrin.
7. Wash with 2 mL FACS buffer.
8. Centrifuge and aspirate supernatant.
9. Resuspend cells with 0.5–2 mL FACS buffer. Place samples in 12 x 75 mm Falcon® tubes and analyze by flow cytometry as soon as possible (within 1 hour). Alternatively, samples can be fixed with 2% paraformaldehyde fixation buffer and stored at 4°C in the dark for up to one week before flow cytometry analysis.

III. Procedure for Intracellular Antigens Analysis

1. Collect 1–3 x 105 cells per sample, wash with PBS.
2. Fix the cells by adding 100 µL of fixation buffer to cell pellet. Vortex and incubate at room temperature for 20 minutes.
3. Add 1 mL of permeabilization buffer to each tube. Centrifuge and aspirate supernatant.
4. Resuspend cells in 100 µL of permeabilization buffer and incubate at room temperature for 5 minutes.
5. Wash cells with 2 mL FACS buffer. Centrifuge and aspirate supernatant.
6. Perform antibody staining as procedure II from step 3–7.
7. Clear in xylene 2x for 5 minutes.
8. Coverslip with mounting medium (KHH001).

Note: If it is desired to simultaneously analyze surface and intracellular molecules at single-cell level, staining cell-surface antigen first by following procedure A step 1–6, then stain the intracellular antigen by following procedure B. Perform all procedure B steps in dark.

IV. Procedure for Staining DNA for Cell Cycle Analysis

This procedure utilizes ethanol to fix the cells and permeabilize the membrane, which allows the dye (propidium iodide) to enter the cells. Propidium Iodide (PI) is a DNA-binding fluorochrome that intercalates in the double-helix. Ribonuclease-A is used to eliminate the staining of double-stranded RNA.

1. Collect 1–3 x 105 cells per sample. Wash with PBS.
2. Fix cells in 70% ethanol at 4°C for 30 minutes. For proper fixation and to prevent cell aggregation, employ agitation while fixing.
3. Centrifuge fixed cells and resuspend pellet in 1 mL of PBS.
4. Add 100 µL of 200 µg/mL DNase-free, RNaseA and incubate at 37°C for 30 minutes.
5. Add 100 µL of 1 mg/mL propidium iodide (light sensitive) and incubate at room temperature for 10–30 minutes.
6. Place samples in 12 x 75mm Falcon® tubes and analyze by flow cytometry.

Note: Procedure IV can be combined with the procedure II of cell surface antigens analysis. Use only FITC-labeled antibodies for surface antigen, since PI emits in the orange to red region of the spectrum (FITC emits green). Do not fix the cells with paraformaldehyde. Start procedure IV after step 6 of procedure II.