# FACS

**Introduction**

FACS stands for **F**luorescence-**A**ctivated **C**ell **S**orting, a specialized type of Flow Cytometry. In addition to recording the size distribution of cells based on light scattering, fluorophore-conjugated antibodies are used to label and record subsets of cells to separate them based on their type / biochemistry. The instrumentation offers a fast, objective, and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells based on size and fluorescent label. Each cell will display an appropriate fluorescent light emission, consistent with the total component presence in the cell. This emission is counted. Tabulation of counted data, in conjunction with size analysis, enables determination of relative percentages of each specific cellular subset, even when the size of the cell is identical to other subset species.

**Buffers:**

PBS: Phosphate Buffered Saline, pH=7.4

Blocking Buffer: 0.5% BSA in PBS

Fix Buffer: 2-4% paraformaldehyde in PBS

Penetrating Buffer: 90% methanol /10% PBS

All centrifuge steps at 1500-2000 rpm for 5 min

**Procedure:**

1. Collect the cell suspension and adjust the cell concentration to 1-5 x 106 cells/ml.

2. Wash cells in PBS and centrifuge.

3. Resuspend cells in 1 ml fix buffer and incubate at room temperature for 10 min.

4. Wash cells in PBS. Resuspend in 2 ml blocking buffer, then shake slightly and centrifuge. Resuspend in 1 ml blocking buffer and skip steps 5+6 if extracellular target is labelled.

5. Only for intracellular targets: Resuspend cells in 1 ml precooled penetrating buffer and incubate at room temperature for 10 min.

6. Wash cells once with 2 ml blocking buffer and resuspend in 1ml blocking buffer.

7. Incubate cells in blocking buffer for 30 min at room temperature.

8. Add primary antibody at 0.025 mg/ml and incubate for 90 min at room temperature.

9. Wash cells two times with 2 ml blocking buffer.

10. Add FITC-conjugated secondary antibodies and incubate for 40 min at room temperature (For direct labelling using a conjugated primary antibody, skip this step).

11. Wash cells two times with 2 ml blocking buffer.

12. Re-suspend cells in 1 x PBS and analyse on flow cytometer.