

# Flow Cytometry Protocol for PBMC's

## Core Assays

1. CD45/CD14/CD3/CD19
2. CD25/CD4/CD3/CD8
3. CD45RA/CD62L/CD4/CD8
4. CD14/CD64/CD3/CD54
5. CD16/CD3/CD14/CD56
6. IgG/IgM/CD19/CD5

## Methods

*FACS blocking buffer is stored in the glass door fridge.*

1. Add 350ul of FACS blocking buffer to a cell pellet of  $1 \times 10^6$  PBMC and allow to block at room temperature for 20 minutes.
2. Aliquot 50ul of the cell suspension to each staining well (~150,000 cells).
3. Add appropriate antibodies to each well (see experimental panels above and control panels below). Gently vortex.
4. Incubate at room temperature in the dark for 15 minutes.
5. Spin down (3 minutes, 1200 rpm (270g), 4C), remove supernatant.
6. Resuspend cells by gently vortexing.
7. Add 200ul FACS buffer and spin down (3 minutes, 1200rpm(270g), 4C), remove supernatant.
8. Resuspend cells by gently vortexing.
9. Add 200ul PBS and spin down (3 minutes, 1200rpm(270g), 4C), remove supernatant.
10. Resuspend cells by gently vortexing.
11. Add 200ul of 0.8% paraformaldehyde. Mix by gently vortexing.
12. Cover plate with sticky cover and foil and store at 4C.
13. When ready to analyze cells on Flow Cytometer, aliquot cells into FACS tubes. You should have about 200ul of cell suspension in each FACS tube.

## **Controls**

Isotype I: 1ul each of the following

FITC IgG1

PE IgG1

PerCP IgG1

APC IgG1

Isotype II: 1 ul each of the following

FITC IgG1

PE IgG2A

PerCP IgG1

APC IgG2B

Single Stain Controls: Use 5ul each of the following

I. FITC CD3

II. PE CD3

III. PerCP CD3

IV. APC CD3