Generic Cell Surface Staining Procedure

Materials:

COMPtrol Kit (Spherotech #CMIgP-30-2K) – Antibodies
Stained according to manufacturer's directions

1. Add 50 ul beads (orange cap) to each comp tube
2. Add antibody to each tube (from Excel sheet)
3. Incubate for 15-30 min at RT
4. Add 50 ul of Blank beads to each tube and the unstained tube
5. Add 2 ml D-PBS to each tube and centrifuge at 1500 RPM for 5 min
6. Decant supernatant and resuspend in 300 ul D-PBS

ArC Amine Reactive Compensation Kit (ThermoFisher #A10628) – Viability Dye
Stained according to manufacturer's directions

1. Add 1 drop of beads (green top tube) to tube
2. Add 3 ul Live/Dead Blue dye to tube
3. Incubate for 30 min at RT
4. Add 1 drop of Blank ARC beads to tube
5. Add 2 ml D-PBS to each tube and centrifuge at 1500 RPM for 5 min
6. Decant supernatant and resuspend in 300 ul D-PBS

Viability Dye

Live/Dead Fixable Blue Dead Cell Stain (ThermoFisher #L23105)

1. Add 50 µl DMSO to 1 vial of dye to reconstitute
2. Add 50 µl D-PBS (Ca^{2+} Mg^{2+} free) to vial
3. Use 1µl to stain each sample

Note: This procedure will work with other colors of Live/Dead Fixable Dyes

Antibodies

Titrate antibodies prior to staining
Prepare antibody staining cocktail by multiplying the amount of each titrated antibody per sample by the number of samples + 2 extra samples. Add all antibodies into one mixture and then use that mixture to stain each sample.

Cells

Prepare cells by centrifuging out of media and resuspending at 1 x 10^7 cells/ml in D-PBS (Ca^{2+} Mg^{2+} free)

Staining Procedure:

1. Prepare antibody staining cocktail and viability dye
2. Stain compensation beads
3. Add 100 µl cells (1 x 10^6 cells) to 12 x 75 mm polystyrene tubes (Falcon #352058)
4. Add 1 µl Live/Dead Blue dye to cells and incubate for 10 min at room temperature, protect from light
5. Add antibody cocktail to tubes and incubate for 30 min at RT, protect from light
6. Add 2 ml D-PBS (Ca^{2+} Mg^{2+} free) to each tube and centrifuge at 1500 RPM (450g) for 5 min. ***Note: A centrifuge with a swinging bucket rotor works much better than a microcentrifuge.***
7. Decant supernatant and resuspend in 300 µl 1% ultrapure formaldehyde
8. Refrigerate until acquisition