Paraformaldehyde/Saponin Staining Method

1. Isolate cells into FACS tubes at approximately $10^6$ cells.

2. Wash cells with FACS buffer, 1200 rpm x 5 min.

3. Stain cells with extracellular antibodies for approximately 30 minutes using standard protocol.

4. Wash with FACS buffer 1200 rpm x 5 min.

5. Aspirate the supernatant from FACS tubes.

6. Add 100 ul/10$^6$ cell of 1% paraformaldehyde in PBS.

7. Incubate at RT for 10 min.

8. Wash with 1 ml 0.03% saponin (in PBS), spin at 1400 rpm for 5 min.

9. Add 100 ul of 0.3% saponin, 20 ul FBS and 0.5 ug antibody (1 ug for primary cells) or control antibody. Vortex/mix and incubate for 30 min at 4°C.

10. Wash with 0.03% saponin 1400 rpm x 5 min.

11. Add 100 ul 0.3% saponin and then 1 ug of secondary antibody conjugated to fluorochrome (i.e. anti-IgG-FITC). Vortex/mix and incubate for 30 min at 4°C.

12. Wash with 0.03% saponin at 1400 rpm x 5 minutes followed by a second wash in FACS buffer.

NOTES: try to keep the cells on ice after fixation with paraformaldehyde as this will help keep the cells intact and the profiles clean.

Do the wash spins at 4°C.