Intracellular Staining (IFN Gamma, IL-4, etc) Protocol

DO NOT USE THIS PROTOCOL FOR FOXP3 STAINING

Example:

	FL1	FL2	FL3	FL4	FL5
12	Thy1.2	isotype		CD8	CD4
13	Thy1.2	IFN Gamma		CD8	CD4
14	Thy1.2	IL-4		CD8	CD4

1. Equally aliquot cells into FACS tubes in preparation for staining
2. Take cells that are to be stained intracellularly, combine into one FACS tube, and centrifuge. Stain other cells using normal protocol
3. Make incubation media for intracellular tubes while cells are spinning (200µL needed per FACS tube)
    a. Using 1640 complete media, add 0.1µL / 1mL media ionomycin (Sigma # I0634) and 0.0065 µL / 1mL media PMA (Sigma # P8139; for example, I add 0.32µL ionomycin and 0.021µL PMA to 3.2mL 1640 CM*)
    b. Briefly vortex incubation media
4. Once cells spin down, discard supernatant and resuspend in 200µL incubation media per FACS tube (using above example, resuspend in 600µL incubation media)
5. Pipet 200µL into separate wells of a round-bottom (U bottom) 96-well plate (using above example, 3 wells are used)
6. Place plate in cell incubator for one hour
7. Add 1µL of GolgiPlug (BD # 555029) per 1mL media to each well (in example, 0.2µL of GolgiPlug is added to each individual well since each contains 200µL media)
8. Put plate back in incubator for 2.5 hours
9. After time expires, mechanically dislodge cells via pipetting, place cells back in separate FACS tubes, spin, and stain as usual

*CM = Complete Media = RPMI 1640 w/ 10% FBS, 1% Pen/Strep (complete RPMI 1640)