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

*DO NOT USE THIS PROTOCOL FOR FOXP3 STAINING*

Example:

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<tr>
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<th>FL1</th>
<th>FL2</th>
<th>FL3</th>
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<tbody>
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<td>12</td>
<td>Thy1.2</td>
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<td>CD8</td>
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<td>IFN Gamma</td>
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<td>14</td>
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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)