Staining Cells in a 96-well Plate (PBMCs)

1. Prepare a single cell suspension of up to 1x10e7 cells per mL.
2. Process cells with FC block prior to aliquoting out
    a. For Human use FC Block (available from many vendors)
    b. For mouse use CD16/CD32 purified.
3. Add 50uL of cells to each well.
4. Wash the cells by adding 200uL/well of wash buffer to each well.
5. Centrifuge 400-600g for 5 minutes.
6. Add a viability dye and incubate according to that dye's instructions
7. Wash the cells by adding 200uL/well of wash buffer to each well.
8. Centrifuge 400-600g for 5 minutes.
9. Add 50uL of correctly diluted Ab to each of your control wells.
10. Add 50uL of master-mix to each sample well.
11. Mix gently for 2-3 minutes on a plate shaker.
12. Incubate for 20-30 minutes on ice or at 2-8 degrees in the dark.
13. Wash the cells by adding 200uL/well of wash buffer to each well.
14. Centrifuge 400-600g for 5 minutes.
15. Discard supernatant.
16. Repeat wash – Steps 13-15
17. Resuspend cells in 100uL staining buffer and 100uL of fixation buffer.
18. Analyze samples as soon as possible but no later than 3 days.

Fixation Buffers:

BD – Cytofix 554655
Biolegend – Fixation Buffer 420801
Thermofisher – Fixation buffer 08-8222-49

FC Block:

BD – Human FC Block 564219
BD – Mouse FC Block 553141S
Biolegend – Human FC Block 422302
Biolegend – Mouse FC Block 101320
Thermofisher – Human FC Block 14-9161-73
Thermofisher – Mouse FC Block 14-0161-82