**AURORA TRAINING EXERCISE #2**

1. **For each assay specify the needed unstained control as well as best options for reference controls**

Example : T-cell Panel for human whole blood (WB, lyse wash assay)

| Marker | Fluor | Level of Expression /Cell Frequency | Control: Beads or Cells | Control Stained With |
|---|---|---|---|---|
| CD3 | FITC | High / high | cells | Any bright FITC reagent |
| CD4 | BV510 | High/ high | cells | Any bright BV510 reagent |
| CD8 | APC H7 | High /high | cells | CD8 APC H7 same lot |
| CD127 | PE CF594 | Intermediate/high | cells | CD127 PE CF594 same lot |
| CD25 | APC | Low/ low | beads | Any brigth APC reagent |
| PD-1 | PE Cy7 | Low / low | beads | PD-1 PE Cy7 |
| unstained | | | WB, lyse wash | |

**a.** B-cell panel for human PBMCs

| Marker | Fluor | Level of Expression / Cell Frequency | Control: Beads or Cells | Control Stained With |
|---|---|---|---|---|
| CD19 | BV510 | High/ intermediate | | |
| IgD | BV421 | High/ intermediate | | |
| CD27 | BV785 | Intermediate/ high | | |
| CD62L | BV605 | High/ high | | |
| CD38 | Pacific Blue | High/intermediate | | |
| IgM | BV650 | High/ intermediate | | |
| IgG | B711 | Low/ low | | |
| unstained | | | | |

**b.** Intracellular Cytokine Staining/ Proliferation Assay cultured PBMCs

| Marker | Fluor | Level of Expression / Cell Frequency | Control: Beads or Cells | Control Stained With |
|---|---|---|---|---|
| CFSE | | High/high | | |
| CD3 | PE CF594 | Low/ high | | |
| CD4 | APC | Low/high | | |
| CD8 | APC R700 | Intermediate/high | | |
| IFNg | efluor 450 | Intermediate/ int. | | |
| IL-4 | PE Cy7 | High/low | | |
| IL-17 | PerCP e710 | Low/low | | |
| viability | Aqua Amine Dye | high/low | | |
| unstained | | | | |

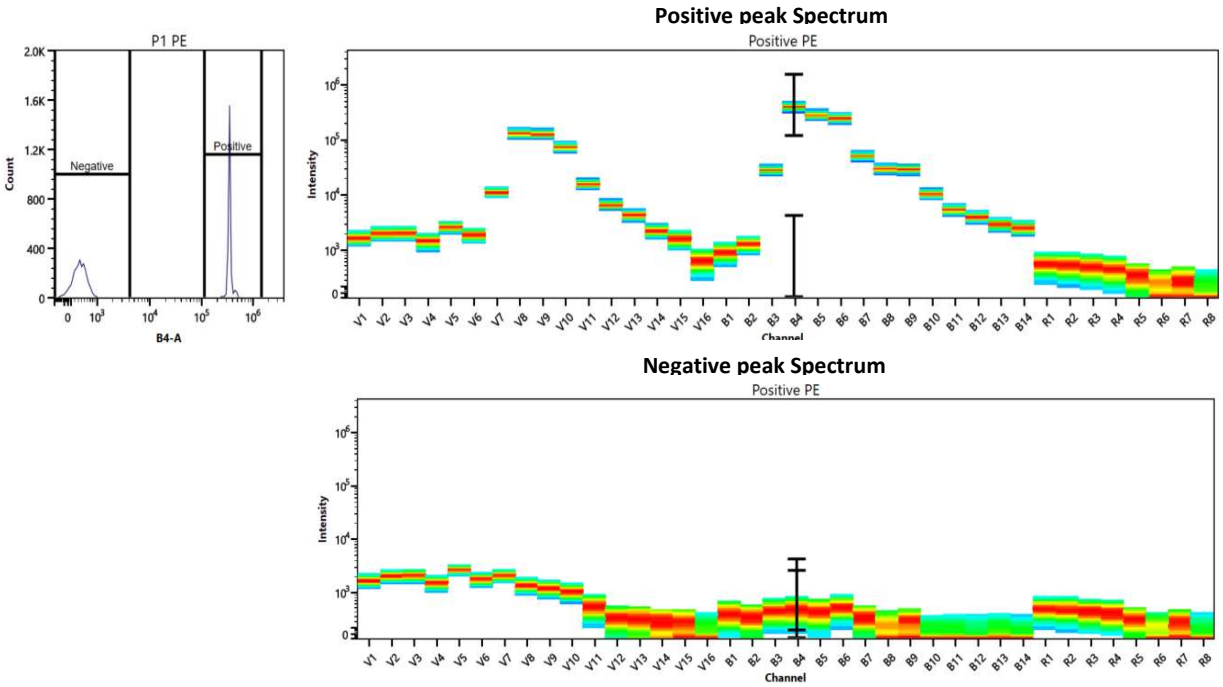
2. **You are planning to perform an assay for detection of FOXP3 and to follow the protocol below. Please write down the protocol you will follow (step by step) to stain beads to be used as controls for surface antigens AND for intracellular antigens**

**FOXP3 Intracellular Staining Procedures:**

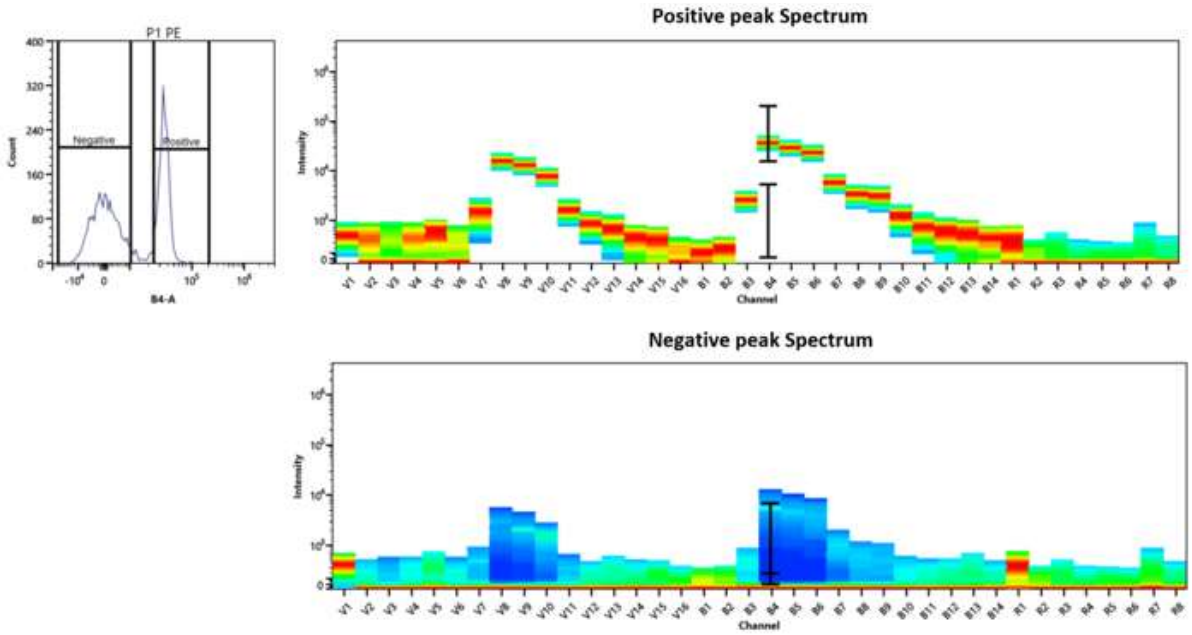
1. Perform cell surface staining as described in BioLegend's Cell Surface Immunofluorescence Staining Protocol.
2. Add 1 ml of 1X BioLegend's FOXP3 Fix/Perm solution to each tube, vortex and incubate at room temperature in the dark for 20 minutes, then spin down the cells and remove the supernatant.
3. Wash once with cell staining buffer (Cat. No. 420201) by spin at 250Xg for 5 minutes and remove the supernatant.
4. Wash once with 1ml 1X BioLegend's FOXP3 Perm buffer.
5. Re-suspend cells in 1ml 1X BioLegend's FOXP3 Perm buffer, incubate at room temperature in the dark for 15 minutes, spin down cells and discard the supernatant, then resuspend the pellet in 100 ul of 1X BioLegend's FOXP3 Perm buffer.
6. Add appropriate amount of flurochrome conjugated anti-FOXP3 antibody and incubate at room temperature in the dark for 30 minutes.
7. Wash twice with cell staining buffer, and resuspend in 0.5ml cell staining buffer then analyze with flow cytometer with appropriate instrument setting.

3. **Please comment on the quality of the reference controls below**

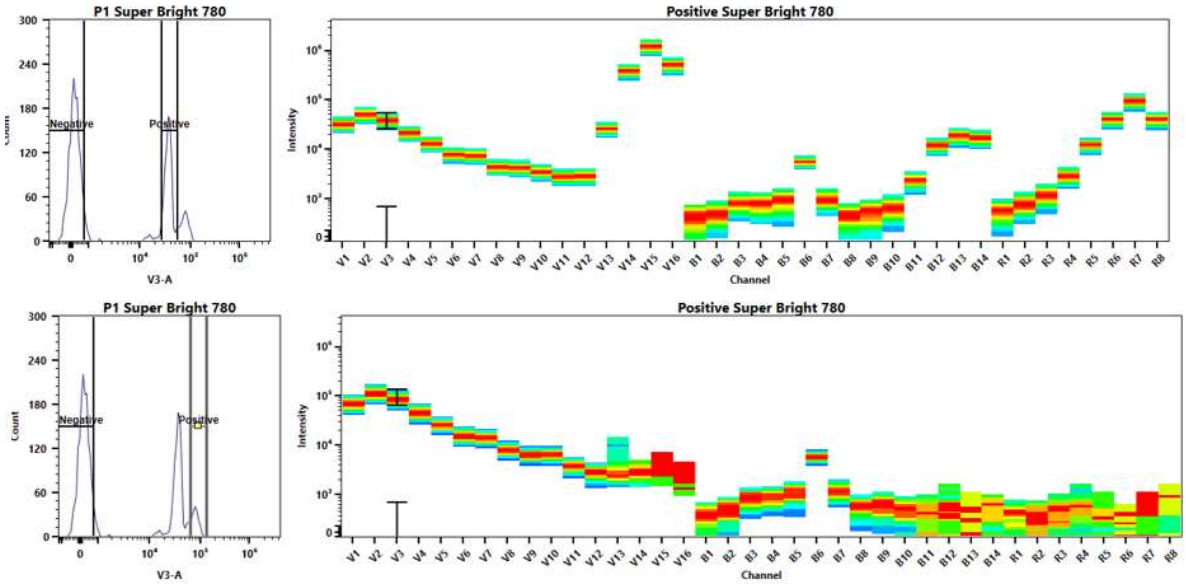
a. PE control




b. PE control



c. Super Bright 780 control




d. Alexa 532 control