**PBMC Staining for Flow Cytometry**

1. Count fresh cells after PBMC isolation.
2. Plate 107 cells in a round-bottom plate.
3. Spin at 1200 rpm for 5 min. Discard supernatant.
4. Wash plate with 200 ul of PBS. Spin at 1200 rpm for 5 min and discard supernatant.
5. Resuspend cells in 100 ul of antibody Panel 1 – Mix 1 diluted in PBS:

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| **Panel 1 – Mix 1** | | |
| **Marker** | **Channel** | **Dilution** |
| Live/Dead | Amcyan | 1:500 |
| CD3 | Pacific Blue | 1:25 |
| CD14 | Pacific Blue | 1:25 |
| CD19 | Brilliant Violet 650 | 1:25 |
| CD20 | Brilliant Violet 570 | 1:25 |
| CD27 | Brilliant Violet 785 | 1:25 |
| CD38 | PerCP-Cy5.5 | 1:25 |
| IgD | Pe-Cy7 | 1:25 |
| ICOS-Ligand | PE | 1:50 |

1. Incubate at room temperature for 45 min.
2. Add 100 ul of PBS and spin at 1200 rpm for 5 min.
3. Wash plate with 200 ul of PBS and spin at 1200 rpm for 5 min. Discard supernatant.
4. Add 200 ul of 1X FOXP3 Fix/Perm solution (BioLegend) and incubate at room temperature for 20 min.
5. Spin at 1200 rpm for 5 min. Discard supernatant.
6. Wash plate with 200 ul of 1X FOXP3 Perm buffer (BioLegend). Spin at 1200 rpm for 5 min and discard supernatant.
7. Add 200 ul of 1X FOXP3 Perm buffer and incubate at room temperature for 15 min.
8. Spin at 1200 rpm for 5 min. Discard supernatant.
9. Add 100 ul of antibody Mix 2, dilute in FOXP3 Perm buffer:

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| **Panel 1 – Mix 2** | | |
| **Marker** | **Channel** | **Dilution** |
| IgM | APC | 1:50 |
| IgG | Alexa 700 | 1:50 |
| FoxP3 | FITC | 1:50 |

1. Incubate at 4oC for 30 min.
2. Add 100ul of FOXP3 Perm buffer and spin at 1200 rpm for 5 min. Discard supernatant.
3. Wash plate twice with 200 ul of PBS. Spin at 1200 rpm for 5 min and discard supernatant.
4. Resuspend cells in 200 ul of BD Stabilizing Fixative and store at 4oC until ready to run samples.