

Titration PD-1 into AIMs for CytoFlex LX

- Basically, make sure you titrate the antibody using a condition where you will see that marker, so you can see positive and negative populations. In the case of PD-1, Cytostim stimulation should upregulate PD-1 so I can find positive and negative populations (ish)
- Note: if you have a viability dye, include staining for that as part of your panel when you titrate.
- Note: I typically include all my panel markers when I titrate something new into the panel, so I can see how it will actually look when in there with the other colors.
- At the very least, you need whatever markers are upstream of the marker you're titrating. For instance if I want to look at PD-1 on T cells, I should include my T cell markers in the titration so I can look at PD-1 separation in this population of interest
- The below is just a simplified example

Specificity	Conjugation	Clone	Supplier	Cat number
CD3	Brilliant Violet 510	UCHT1	BD Biosciences	563109
CD4	Brilliant Blue 700 (BB700)	SK3	BD Biosciences	566392
CD25	PECy7	M-A251	BD Biosciences	557741
CD134	PE	ACT-35	BioLegend	350004
CD39	PEDazzle594	A1	BioLegend	328224
CD8	Brilliant Blue 515 (BB515)	RPA-T8	BD Biosciences	564526
CD137	APC	4B4-1	BioLegend	309810
CD69	Brilliant Violet 711 (BV711)	FN50	BioLegend	310944

The panel antibody cocktail to be used for staining, per tube, consists of:

0.5µL CD134-PE
1.0µL CD25-PECy7
1.0µL CD137-APC
1.0µL CD69-BV711
2µL CD39-PEDazzle594
1µL CD3-BV510
1µL CD4-BB700
1µL CD8-BB515
X µl PD-1-BV421
Total mAb volume = 13.5µL

PBS = 26.5uL

Stain Buffer Plus = 10uL

Note: adjust volumes based on your final stain volume, my final stain volume here was 50ul.

Will have the following conditions:

1. 5 ul PD-1
2. 2.5ul PD-1 + 2.5 ul PBS
3. 1 ul PD-1 + 4ul PBS
4. 0.5 ul PD-1 + 4.5ul PBS
5. Cytostim FMO (no PD-1) just add 5ul pbs

Make pre labelled tubes from the master mix, and individually add the PD-1 and extra PBS (if needed) to them. Can then use the antibody cocktail to resuspend cells in wells and transfer them over to the 2ml eppendorfs (5 total).