REAGENTS:

Item	Manufacturer	Order Number
RPMI-1640	Sigma	R0883
Pen/Strep (5000 IU Pen/ 5000ug/mL Strep)	Mediatech	30-001-Cl
L-glutamine (200mM; 29.2 mg/mL)	Mediatech	25-002-Cl
HEPES (1M; 238.3mg/mL)	Mediatech	25-060-Cl
FBS, Heat-inactivated	Sigma	F4135 (a lot tested for here)
PBS	Sigma	D8537
Tetramers or pentamers	Backman Coulter or proimmune	Cat# depends on specificity

Tetramer Staining

1. Count cells to be stained; you will need about 0.2 million clones and 1-2 million PBMCs.

   In addition to cells you plan to analyze, a separate aliquot of the same cells must be set as FMO with all surface and intracellular antibodies except the tetramer/pentamer, especially for small population.

2. Wash cells using **cold** 1% FCS in 1×PBS wash buffer- wash for 7 minutes at 1500rpm. This can be made by adding 5 ml heat inactivated FCS to a new 500 ml bottle of PBS.
3. Resuspend cells in 0.1 ml of wash buffer per tube Transfer the cell suspensions to FACS tubes if you have not already done so.
4. Add tetramer at 1-2 µl for each tube. If tetramers is older than 12 mc add more per staining (this should be titrated before to make sure what concentration of tetramer is optimals for staining). Cover the tubes with aluminum foil.
5. Incubate at RT in the **dark** for about 30 minutes. A shorter incubation time at RT, e.g. 20 min, is usually enough for Class I tetramers. For Class II: incubate 30 min at 37 C
6. Wash 1x with PBS 1% FCS 7 minutes at 1500rpm.
7. Add the correct antibodies such as CD3-FITC 1.5µl, CD8-APC 1.5 ul, then incubate at 4°C for 15 minutes. For small responses, strongly consider addition of an exclusion channel(s) for CD14, CD19 and dead cell viability dye (in this case, begin by viability dye stain)
8. Wash cells once with 3 ml of wash buffer. Spin down at 1500 rpm for 7 minutes, then aspirate down to 100 µl.
9. Fix cells in 1% PFA at 200-300 µl.

10. Ready for FACS analysis the same day. Your results will be worse if you wait.