STANDARD OPERATING PROCEDURE				
Ragon Institute of MGH, MIT and Harvard	Tetramer Staining		**SOP#**	TC-SOP-013
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I. PURPOSE

The purpose of this procedure is to establish and outline the requirements for attire and personal protective equipment (PPE) for Ragon Institute laboratories.

II. SCOPE

This procedure applies to all employees, students, contractors and visitors that enter and/or work in the Ragon Institute laboratories.

III. RESPONSIBILITIES

A. The Ragon Institute **Director and all Laboratory Principal Investigators** are responsible for the overall implementation of this procedure and ensuring compliance.
B. **Environmental Health & Safety (EH&S)** is responsible for periodically reviewing the application and maintenance of this procedure, and initiating any updates to this procedure.
C. **All employees, students, contractors and visitors** are required to follow this procedure. Non-compliance with this procedure will result in the assignment of a corrective action plan.
D. **The Partners Institutional Biosafety Committee, Ragon Laboratory Managers and/or EH&S** are responsible for outlining additional PPE beyond what is stated in Section IV. (Procedure) if warranted through a regulatory requirement or industry best-practice.

IV. PROCEDURE

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 (Ragon tested lot)
PBS	Sigma	D8537
Tetramers or pentamers	Backman Coulter or proimmune	Cat# depends on specificity
Dead cell viability	Invitrogen	L23105

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. After wash. decant supernatant leaving approximately 1-300 µl of PBS with the cells. Vortex FACS tubes to mix.
3. Add 200 µl dead cell viability dye to each FACS tube and then vortex to mix *Remember to first make 1:1000 dilution of the cell viability dye before adding to FACS tube, eg: 1µl stock viability dye per 1mL PBS , therefore 1.8ml = 1.8 µl of stock viability dye to be added, 2ml = 2 µl, etc. Vortex to mix solution together. After addition of dead cell viability dye, incubate in the dark for 15 min.
4. Wash cells using **cold** 1% FCS in 1×PBS wash buffer- wash for 7 minutes at 1500rpm. After wash. decant supernatant leaving approximately 1-300 µl of PBS with the cells. Vortex FACS tubes to mix.
5. Add tetramer at 1-2 µl for each tube. If tetramers is older than 12 months 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. Incubate in the dark at RT for 30 min. A shorter incubation time at RT, eg. 20 min is usually enough for Class I tetramers. Class II tetramers, incubate 30 min @ 37C.
6. Wash cells using **cold** 1% FCS in 1×PBS wash buffer- wash for 7 minutes at 1500rpm. After wash decant supernatant leaving approximately 1-300 µl of PBS with the cells. Vortex FACS tubes to mix.
7. Add the correct antibodies such as CD3-FITC 1.5µl, CD8-APC 1.5 ul, then incubate in the dark @ 4°C . 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 using **cold** 1% FCS in 1×PBS wash buffer- wash for 7 minutes at 1500rpm. After wash decant supernatant leaving approximately 1-300 µl of PBS with the cells. Vortex FACS tubes to mix.
9. Fix cells in 1% PFA at 200-300 µl. Vortex to mix. Incubate at RT for 15 min.
10. Wash cells using **cold** 1% FCS in 1×PBS wash buffer- wash for 7 minutes at 1500rpm. After wash decant supernatant leaving approximately 1-300 µl of PBS with the cells. Vortex FACS tubes to mix. Keep cells on ice or 4°C until ready for FACS analysis.
11. Ready for FACS analysis the same day. Results will be worse if you wait.