BENAROYA RESEARCH INSTITUTE
Virginia Mason

Tetramer Lab Core
Standard Protocols

Direct ex vivo staining

1. Resuspend PBMCs in T cell medium at a density of 150 million/mL.
2. Aliquot 30 million cells into each FACS tube – save some for compensation.
3. Add dasatinib to a final concentration of 50 nM. Incubate at 37°C for 10 minutes.
4. Add 4-6 µl tetramers into each FACS tube. Incubate in the dark at room temperature for 2 h.
5. Wash once with 2 mL running buffer. Decant the supernatant and leave ~ 150 µl in each FACS tube.
6. Add 40 µl anti-PE or anti-APC microbeads into each tube.
7. Incubate in the dark at 4°C for 20 minutes.
8. Wash once with 2 mL running buffer.
9. Decant the supernatant. Leave 150 µl in the tube and add 900 µl running buffer.
10. Take aside 10 µl of cell solution and transfer into a new tube containing 1mL running buffer. This is your “pre-enrichment” tube.