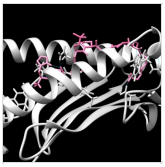

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Detection of Rare, Antigen-specific Human T cells with HLA Tetramers

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

How I stain human T cells with tetramers following the guidelines from Sewell's group: https://pubmed.ncbi.nlm.nih.gov/26076649/

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ABSTRACT

How I stain human T cells with tetramers following the guidelines from Sewell's group: https://pubmed.ncbi.nlm.nih.gov/26076649/

Staining

1 Harvest cells of interest (PBMC, purified T cells, BMMC) either from culture or from frozen stock

2 Wash cells in PBS and resuspend in 50nM Dasatinib in PBS. Choose a volume appropriate with your number of samples in the next step. You will need 50uL of sample per tetramer. Incubate 37C for 30-60m.

3 Add 1uL directly of PE-conjugated tetramer to 50uL cells (still in Dasatinib from step 3). Incubate 30m on ice. The number of cells required will depend on the frequency of your population of interest. It is unlikely that your cells will be too dense at 50uL unless you are staining >50M lymphocytes.

4 Wash samples twice with FACS buffer (we use PBS + 2mM EDTA + 0.5% BSA + 0.1% w/v sodium azide). For multiple samples, performing washes in v-bottom plates is more convenient than tubes. Perform all spin steps at 860g, 4C, 4min.

5 After last spin, resuspend cells in 50uL per sample of anti-PE (diluted 1:100 in FACS buffer; clone PE001). Incubate 30m on ice.

6 Wash twice with FACS. Resuspend in 50uL PE-conjugated Goat anti-Mouse Ig (diluted 1:100 in FACS buffer; assuming you used PE001, a mouse isotype antibody). Incubate 30m on ice.

7 Wash twice with FACS. Resuspend in 50uL of surface stain (e.g. CD3, CD8, CD4, as well as a dump channel including CD16 CD19 CD56 CD14. Differentiation markers can be included as well such as CD62L, CD45RA/RO, CCR7). Incubate 30m on ice.

8 Wash twice with FACS. After last wash, if using 7AAD as live/dead, resuspend cells in 7AAD diluted 1:100 in FACS buffer. Otherwise resuspend in FACS buffer. Strain cells and acquire.

Acquisition

9 If looking for low frequency populations, collect as many events as possible.

10 Gate on live, singlet, dump channel-negative, aggregate-negative CD3+ cells before looking at your Tetramer+ve population. When looking for rare populations, do not be generous with singlet, live/dead, scatter, or phenotypic gates. It is crucial to exclude noisy outliers such as fluorophore aggregates. Exercise caution when choosing to include CD4+CD8+ over single positives.