HLA class I tetramer staining of Human T cells

Materials and equipment

Fluorophore-labeled HLA class I tetramer(s)
Fluorophore-labeled antibodies against phenotypic markers (CD3, CD8, and other optional markers)
96-well U-bottom plate
FACS buffer: PBS with 1%BSA (or FCS) and 0.1% NaN_3.
Centrifuge with a plate rotor
Flow cytometer

Recommendations

Please note that the staining intensity can vary between tetramer specificities, hence the tetramer concentration should be titrated the first time a specific tetramer is used.
Note, it may be an advantage to stain for the same tetramer specificity with two different fluorochrome labels. It gives a more accurate definition of the tetramer positive population. It also allows for analysis of more than one T cell specificity in the same cell sample. Using various fluorochrome labeled tetramers each specificity can be defined by its unique two fluorochrome combination.

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1. Prepare the cells of interest. For PBMC use 1-2*10^6, for cell lines use 2-4*10^5
2. Transfer the cells to a 96-well U-bottom plate: Adjust the volume to 200µl with FACS buffer
3. Spin the plate at 700g for 3min. - flip out the supernatant in one smooth move.
4. Tetramer staining: Dilute the tetramer to 30 nM in FACS buffer and resuspend the pellet in 40 µl of this dilution, and incubate in the dark at RT for 20 min.
5. Wash once in cold FACS buffer.
6. Spin the plate at 700g for 3min. - flip out the supernatant in one smooth move.
7. Co-stain with surface antibodies (CD8, CD3, other phenotype markers) prepare the antibody cocktail based on optimal staining concentration of each reagent.
8. Incubate in the dark at 4°C for 30 min.
9. Wash twice in cold FACS buffer.
10. Resuspend in FACS buffer and analyze in a Flow Cytometer.

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