

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% NaN₃.
Centrifuge with a plate rotor
Flow cytometer

Recommendations

Since, the staining intensity can vary between tetramer specificities, the tetramer concentration should be titrated the first time a specific tetramer is used.

Note, it can be some advantages 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.

HLA class I tetramer staining of human T cells

1. Prepare the cells of interest. For PBMC use $1-2 \times 10^6$, for cell lines use $2-4 \times 10^5$
2. Transfer the cells to a 96-well U-bottom plate: Adjust the volume to 200 μ l 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 30nM in FACS buffer and resuspend the pellet in 40 μ l 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.