Materials and equipment

Fluorophore-labeled HLA class II tetramer(s)
Fluorophore-labeled antibodies against phenotypic markers (CD3, CD4, and other optional markers)
96-well U-bottom plate
Cell culture media
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 staining 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.
Often antigen specific CD4+ T cells are of very low frequency and can be difficult to detect directly ex vivo. A prior enrichment or short 7-day in vitro stimulation is recommended for such detection.

HLA class II tetramer staining of human T cells

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 in 30µl media to a 96U-well U-bottom plate.
3. Spin the plate at 700g for 3min. - flip out the supernatant in one smooth move.
4. Tetramer staining: Dilute the HLA-II tetramer to 30-60nM in media and add 30µl of this dilution to the cells. This gives a final staining concentration of 15-30nM
5. Incubate in the dark for 1h at 37°C.
6. Wash once in cold FACS buffer.
7. Spin the plate at 700g for 3min. - flip out the supernatant in one smooth move.
8. Co-stain with surface antibodies (CD4, CD3, other phenotype markers) prepare the antibody cocktail based on optimal staining concentration of each reagent.
9. Incubate in the dark at 4°C for 30 min.
10. Wash twice in cold FACS buffer. Resuspend in FACS buffer and analyze in a Flow Cytometer.

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