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Tetramer Staining Protocol

Tetramer staining assays are used to detect and quantify antigen-specific T cells. Native peptide MHC complexes (pMHC) and T cell receptors interact with affinities that are too low to be detected in flow cytometry (FACS). Immunitrack produces tetramers of monomeric biotinylated pMHC complexes; these have increased affinity for T cell receptors, allowing FACS detection of antigen-specific T cells.

Reagents Needed

- Tetramers in phycoerythrin (PE) or allophycocyanin (APC). We recommend storing tetramers at a stock concentration of 1000 µg/mL at -20 °C.
- Surface marker panel containing antibodies towards T cell markers: CD3, CD4, and CD8.
- Live/dead stain at concentration recommended by supplier. Immunitrack uses LIVE/DEAD Fixable Dead Cell Stains from Thermo Fisher.
- FACS buffer (PBS, 0.5 % BSA, 0.02 % sodium azide).

Protocol

1. Prepare cells. If using fresh cells: pellet the cells by centrifuging at 300 x g for 5 mins at room temperature, remove the supernatant and then dissolve the pellet in 12 mL FACS buffer (FB). If using frozen cell pellets: thaw the pellets in a water bath warmed to 37 °C for 1 min, and then add 12 mL FB.
2. Pellet the cells in the FB by centrifuging at 300 x g for 5 mins at room temperature. Remove the supernatant and resuspend the pellet in 100 µL FB. Count the cells and dilute as necessary in FB to an approximate density of 10^5-10^6 cells per 100µl, ensuring that you have 100 µL of cells for every well.
3. Add 100 µL of the cell suspension prepared in Step 2 to each well of a v-shaped plate and spin down cells at 300 x g for 5 min.
4. Prepare a Live/Dead solution in FB at a 1:1000 ratio.
5. Next, prepare a tetramer mix by combining 24 µL of the Live/Dead FB dilution from Step 4 with 1 µL tetramer for every sample, to yield a final volume of 25 µL per sample and a final tetramer concentration of 10 µg/mL.
6. Mix and add the 25 µL tetramer mix from step 5 to each well containing cells and incubate at 37 °C for 15 minutes.
7. Mix surface marker antibodies (e.g. CD3, CD8 and CD4) and to this, add FB buffer to a total volume to 25 µL per sample.
8. Add 25 µL of the surface marker mix prepared in Step 7 to the cells in the plate and mix gently by pipetting.
9. Incubate the mixture for 20 minutes 4 °C (i.e. in the fridge).
10. Wash the cells with 150 µL as follows: centrifuge at 300 g for 5 minutes, remove the supernatant, and resuspend in 100 µL FB. Analyse this cell suspension directly on a flow cytometer.

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Figure 1: Example of a representative tetramer staining experiment after flow cytometry analysis.

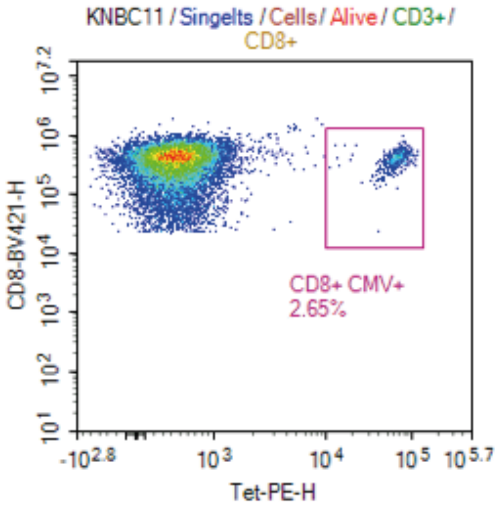

Figure 1: Flow cytometry analysis of a representative tetramer staining of $5x10^5$ peripheral blood mononuclear cells (PBMCs) from a healthy blood donor. The cells were gated for singlets, cell population, live cells, CD3+ cells and CD8+ cells. The CD8+ cell population can be seen in the Brilliant Violet 421 channel and the tetramer cell population is seen in the PE channel. The analysis shows that the donated blood has a population of 2.65 % CD8+CMV+ (NLVPMVATV) cells.