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MBL International Corporation

MHC tetramer staining with various human CD8 clones

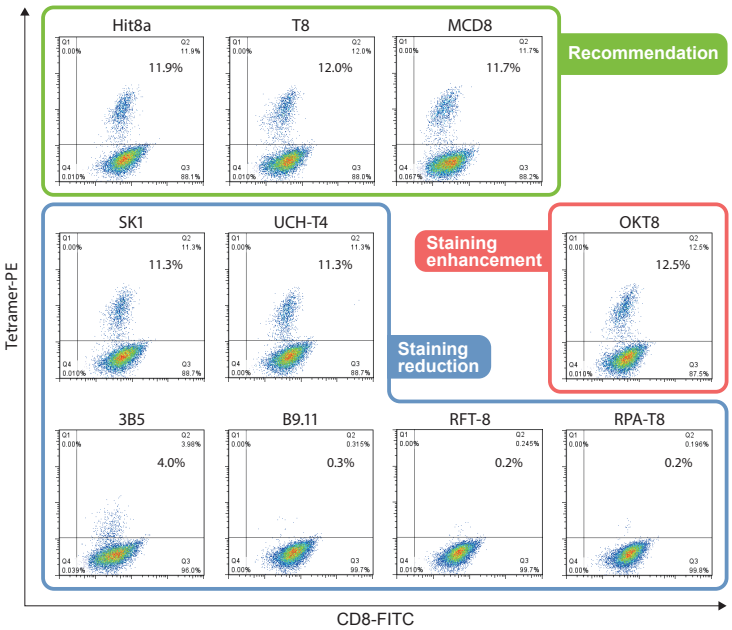
When staining T-cells with an MHC tetramer and an anti-CD8 antibody in flow cytometry, it is crucial to choose the correct CD8 clone for best results. The wrong CD8 clone may lead to undesired reactions for the MHC tetramer binding to the TCR. either enhancing or reducing the tetramer reaction*.

We strongly recommend clones Hit8a, SFCI21Thy2D3 (T8), or MCD8 for human MHC tetramer staining. Please see the product datasheet to ensure proper protocols are used.

Staining differences among human CD8 antibody clones

We compared the effects of 10 different anti-CD8 antibody clones with MBL's MHC tetramer staining. It has been shown that clone OKT8 may enhance tetramer staining*.

*Reference: Campanelli R, et al. Int. Immunol. 14, 39-44 (2002)



※The value in the top right quadrants of the figure indicates the percentage (%) of CD8⁺ tetramer⁺ cells.

MHC tetramer and anti-CD8 antibody staining

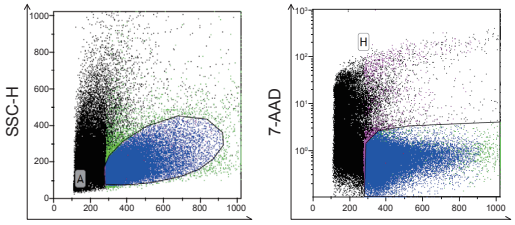
Flow cytometry analysis was conducted using two MHC tetramers and two CD8 clones.

Protocol

1. Prepare cells according to established procedures. Cells should be resuspended at aconcentration of 5 x 10^6 cells/mL 200 µL of sample is required for each Tetramer determination.
2. To each 12x75 mm test tube add 10 µL of MHC Tetramer and any additional antibodies (e.g. anti-CD8).
3. Add 200 µL cells into each test tube (e.g. 1 x 10^6 cells per tube).
4. Vortex gently.
5. Incubate for 30 minutes at room temperature protected from light.
6. Add 3 mL of PBS.
7. Centrifuge tubes at 400 x g for 5 minutes.
8. Aspirate or decant the supernatant.
9. Resuspend the pellet in 500 µL of PBS with 0.5% formaldehyde. (62.5 µL Fixative Reagent / 1 mL PBS).
10. Store prepared samples at 4°C protected from light for a minimum of 1 hour (maximum 24 hours) prior to analysis by flow cytometry.

Gating

7-AAD-negative lymphocytes were gated by FSC/7-AAD plot after FSC/SSC parameter analysis.

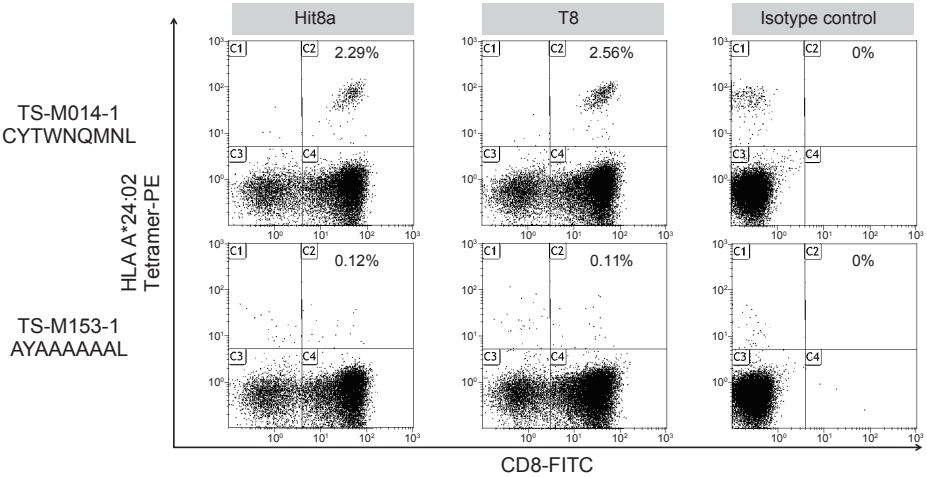


Results

Cells were co-stained with MBL MHC tetramer and anti-CD8 antibodies (clones Hit8a and T8). CD8+ tetramer+ cells were observed clearly.

The following antibodies were used in this assay:

- Anti-CD8a (Human) mAb-FITC (Code No. K0226-4, clone Hit8a)
- CD8-FITC Conjugated Antibody (Beckman Coulter, clone SFCI21Thy2D3 “T8”)
- Mouse IgG1(isotype control) -FITC (Code No. M075-4)



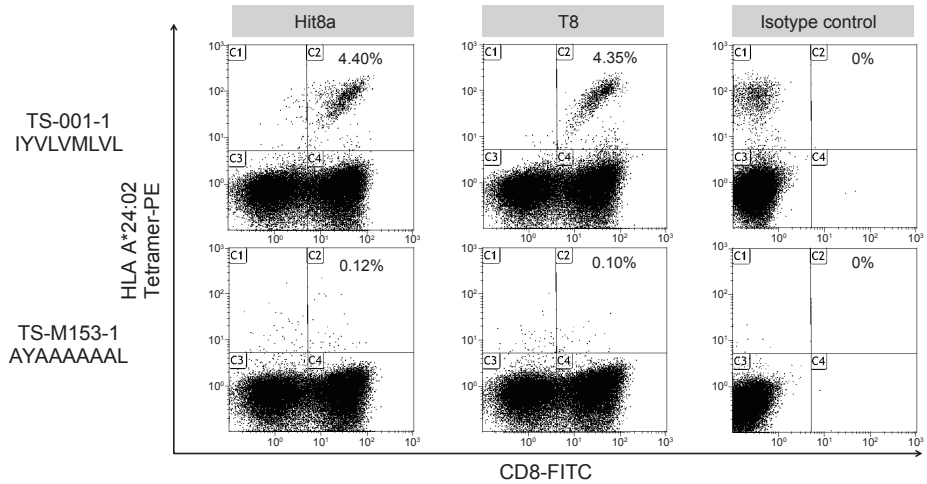
Example 1

Positive Tetramer

T-Select HLA-A*24:02 modified WT1 Tetramer-CYTWNQMNL-PE (Code No. TS-M014-1)

Negative Tetramer

HLA-A*24:02 Control Tetramer-AYAAAAAAL-PE (Code No. TS-M153-1)



Example 2

Positive Tetramer

T-Select HLA-A*24:02 EBV LMP2 Tetramer-IYVLVMLVL-PE (Code No. TS-M001-1)

Negative Tetramer

HLA-A*24:02 Control Tetramer-AYAAAAAAL-PE (Code No. TS-M153-1)

※The value in the top right quadrants of the figure indicates the percentage (%) of $CD8^+$ $tetramer^+$ cells.

Our recommendation

Code No.	Product name	Clone	Isotype	Application	Size
K0226-4	Anti-CD8a (Human) mAb-FITC	Hit8a	Mouse IgG1 κ	FCM	1.0 mL (100 tests)