

Application Note

MHC tetramer staining with various human CD8 clones

When MHC tetramer staining with anti-CD8 antibody in flow cytometry, you need to choose correct CD8 clone to gain the best results. A wrong CD8 clone may lead to undesired reactions on MHC tetramer binding to the TCR, either enhancing or reducing tetramer reaction*.

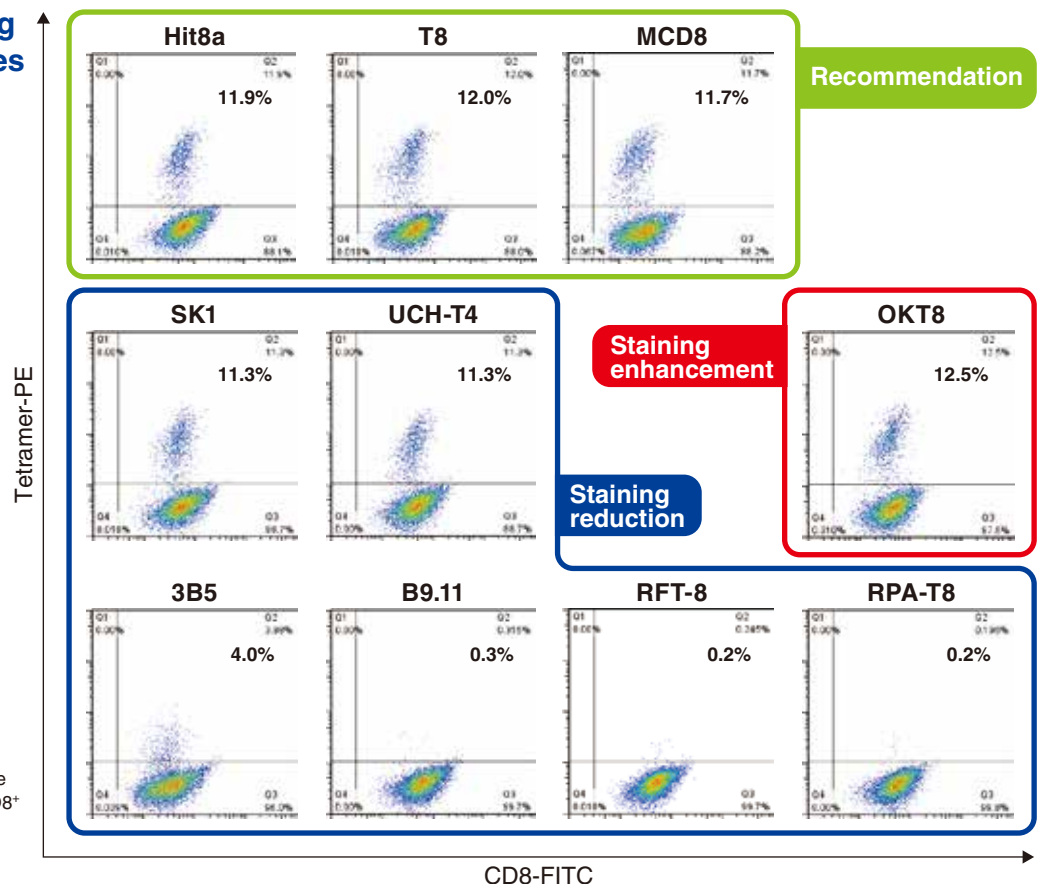
We strongly recommend clones Hit8a, SFC121Thy2D3 (T8), or MCD8 for human MHC tetramer staining. Also make sure the correct protocol is followed.

Staining differences among human CD8 antibody clones

We checked the effects of 10 anti-CD8 antibody clones on MBL's MHC tetramer staining. It has previously 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.



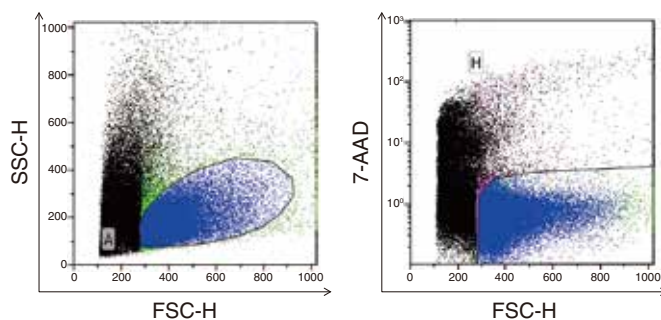
MHC tetramer and anti-CD8 antibody staining

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

Protocol

1. Wash cells with FCM Buffer [2% FCS, 0.05% NaN₃, PBS].
2. Centrifuge at 400 × g for 3 min., and discard the supernatant.
3. Suspend the cells with FCM Buffer. (1.0 × 10⁵ cells/test)
4. Add 10 μL of Clear Back (Code No. MTG-001) to 70 μL of cell suspended buffer and incubate for 5 min. at room temperature.
5. Add 10 μL of MHC tetramer, and incubate for 30 min. at 4°C.
6. Add 10 μL of anti-CD8 antibody (or isotype control) and incubate for 30 min. at 4°C.
7. Wash the cells with 1 mL of FCM Buffer.
8. Centrifuge at 400 × g for 3 min., and discard the supernatant.
9. Suspend the cells with FCM Buffer containing 7-AAD (500 μL), and analyze it.

Gating



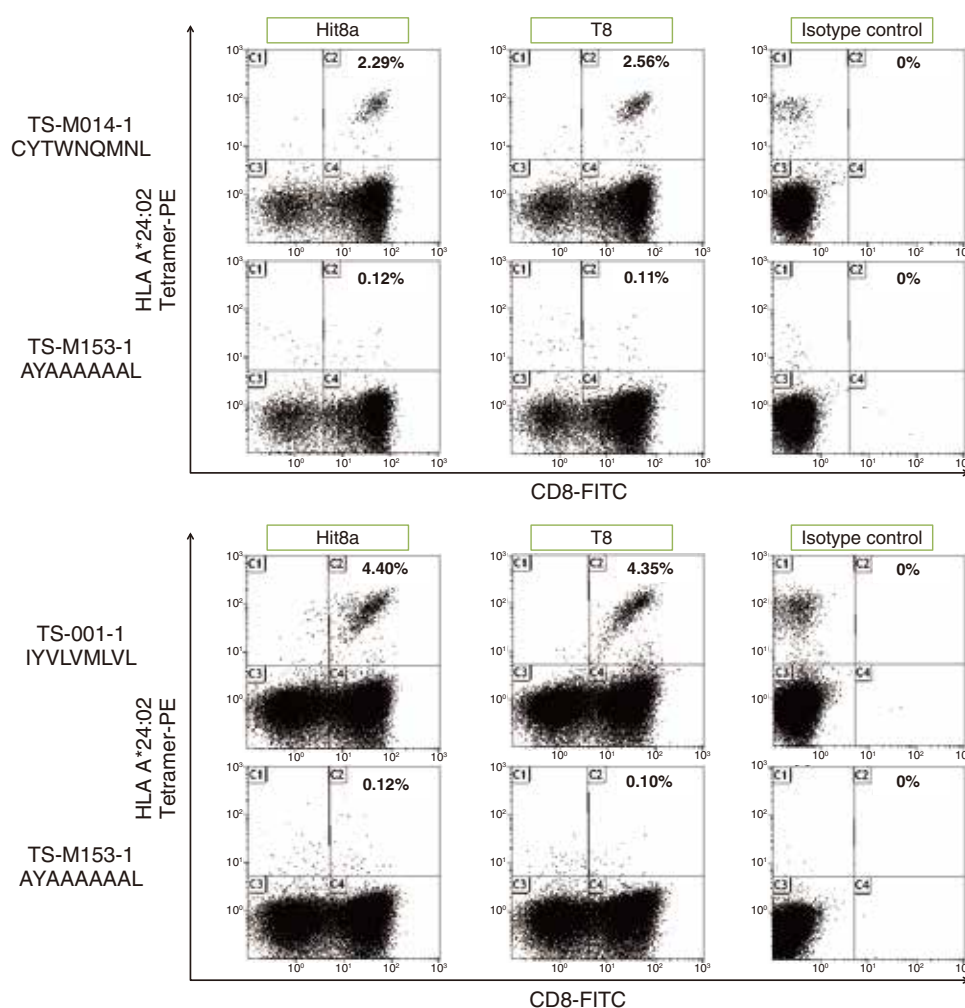
7-AAD-negative lymphocytes were gated by FSC/7-AAD plot after FSC/SSC parameter analysis.

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 SFC121Thy2D3 "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)

Please check our MHC tetramer line-ups!

