

T-Select MHC Tetramer

# HLA-E\*01:01 HLA-A leader<sub>3-11</sub>

## Tetramer-VMAPRTLVL-PE (50 tests)

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### Background

T lymphocytes play a central role in immune system. Total T cell and T cell subset counts are measured by detection of various cell surface molecules. Enumeration of CD8<sup>+</sup> antigen-specific T cells requires cognate recognition of the T cell receptor (TCR) by a class I MHC/peptide complex. This can be done using T-Select MHC class I Tetramers which are composed of four MHC class I molecules each bound to the specific peptide and conjugated with a fluorescent protein. Thus, T-Select MHC Tetramer assays allow quantitation of the total T cell population specific for a given peptide complexed in a particular MHC molecule. Furthermore, since binding does not depend on functional pathways, this population includes specific CD8<sup>+</sup> T cells regardless of functional status. Measurements may be performed in whole blood or isolated lymphocyte/mononuclear cell preparations. In some cases where frequency is low, it may be necessary to perform an *in vitro* cell expansion. Specific cell staining is accomplished by incubating the sample with the T-Select MHC Tetramer reagent, then washing away excess Tetramer. The number of Tetramer positive lymphocytes is then determined by flow cytometry.

Human leukocyte antigen E (HLA-E) is a non-classical class I molecule recognized by natural killer (NK) cells and CD8<sup>+</sup> T cells. HLA-E is expressed in almost all tissues including lung, liver, skin and placental cells. Expression of HLA-E is also detected in solid tumor (e.g., osteosarcoma and melanoma). HLA-E complexed with peptides can bind to TCR expressed on CD8<sup>+</sup> T cells, resulting in the T cells activation. HLA-E/peptide complex is also known to bind CD94/NKG2 receptor expressed on NK cells and CD8<sup>+</sup> T cells. CD94 can pair with several different isoforms of NKG2 to form receptors with potential to either inhibit (NKG2A, NKG2B) or promote (NKG2C) cellular activation.

HLA-E exhibits very low level of polymorphism. Only two alleles of HLA-E, HLA-E\*01:01 and HLA-E\*01:03, have been found. The two alleles are found at nearly equal frequencies in diverse populations and differ only at amino acid position 107 of the protein.

This Tetramer reagent comprises human class I HLA-E\*01:01 and epitope peptide derived from the HLA-A leader, and it can detect HLA-E\*01:01-restricted HLA-A leader<sub>3-11</sub>-specific NK cells and CD8<sup>+</sup> T cells by flow cytometry.

HLA-E preferably binds to a peptide derived from amino acid residues 3–11 of the leader sequences of most HLA-A, -B, -C, and -G molecules, but cannot bind its own leader peptide. Under physiological conditions, the engagement of CD94/NKG2A with HLA-E, loaded with peptides from the HLA class I leader sequences, usually induces inhibitory signals. Cytomegalovirus (CMV) utilizes this mechanism for escape from NK cell immune surveillance via expression of the UL40 glycoprotein, mimicking the HLA-A leader<sub>3-11</sub>. However, it is also reported that CD8<sup>+</sup> T cells can recognize HLA-E loaded with the UL40<sub>15-23</sub> peptide derived from CMV Toledo strain and play a role in defense against CMV. A number of studies revealed several important functions of HLA-E in infectious disease and cancer.

A Tetramer, which is constructed with the same allele (HLA-E\*01:01) of interest and an irrelevant peptide, may be used as a negative control Tetramer.

**HLA Restriction:** HLA-E\*01:01

### Origin and Sequence of This Epitope

HLA-A\*02, A\*24 leader (3-11 aa, VMAPRTLVL).

### References for This Product

- 1) Braud VM, *et al. Nature* **391**: 795-799 (1998)
- 2) Lee N, *et al. PNAS* **95**: 5199-5204 (1998)
- 3) Tomasec P, *et al. Science* **287**: 1031-1033 (2000)
- 4) Heinzl AS, *et al. J Exp Med* **196**: 1473-1481 (2002)
- 5) Miller JD, *et al. J Immunol* **171**: 1369-1375 (2003)
- 6) Pietra G, *et al. PNAS* **100**: 10896-10901 (2003)
- 7) Strong RK, *et al. JBC* **278**: 5082-5090 (2003)
- 8) Salemo-Gonçalves R, *et al. J Immunol* **173**: 5852-5862 (2004)
- 9) Derré L, *et al. J Immunol* **177**: 3100-3107 (2006)
- 10) Monaco EL, *et al. Neoplasia* **13**: 822-830 (2011)
- 11) Weder P, *et al. Results in Immunology* **2**: 88-96 (2012)

## Reagents

500 µL liquid - 10 µL/test

The Tetramer is dissolved in an aqueous buffer containing 0.5 mM EDTA, 0.2% BSA, 10 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 0.09% NaN<sub>3</sub>.

## Conjugates

Streptavidin-Phycoerythrin (SA-PE)

Excites at 486-580 nm

Emits at 586-590 nm

## Storage Conditions

Store at 2 to 8°C. Do not freeze. Minimize exposure to light. The expiration date is indicated on the vial label.

## Evidence of Deterioration

Any change in the physical appearance of this reagent may indicate deterioration and the reagent should not be used. The normal appearance is a clear, colorless to pink (SA-PE).

## Usage

This reagent is for use with standard flow cytometry methodologies.

## High Specificity

The T cell surface CD8 enhances T cell antigen recognition by binding to HLA class I molecules. Therefore, MBL produced T-Select MHC class I human Tetramers with one point mutation at the HLA α3 domain known to alter the interaction with CD8. These mutated Tetramers showed a greatly diminished nonspecific binding but retained specific binding. Alterations of CD8 binding by mutation of the MHC greatly improved the specificity of MHC-peptide multimers, thus providing efficient tools to sort specific human T cells for immunotherapy. (French application Number; FR9911133)

## References for T-Select MHC Tetramer

Altman JD, *et al. Science* **274**: 94-96 (1996)  
McMichael AJ, *et al. J Exp Med* **187**: 1367-1371 (1998)  
Bodinier M, *et al. Nat Med* **6**: 707-710 (2000)

## Statement of Warnings

1. This reagent contains 0.09% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and material coming in contact with them should be handled as if capable of transmitting infection and disposed of with

proper precautions.

3. Never pipette by mouth and avoid contact of samples with skin and mucous membranes.
4. Minimize exposure of reagent to light during storage or incubation.
5. Avoid microbial contamination of reagent or erroneous results may occur.
6. Use Good Laboratory Practices (GLP) when handling this reagent.

## Materials Required But Not Supplied

- 12 x 75 mm polypropylene test tubes
- Transfer pipettes
- Pipettors and disposable pipette tips
- Vortex mixer
- Centrifuge capable of 150 x g or 400 x g
- Aspirator
- PBS
- Red blood cell lysis reagent
- Anti-CD8-FITC, Beckman Coulter, Inc., PN 6603861
- 7-AAD Viability Dye, Beckman Coulter, Inc., PN A07704
- Clear Back (human FcR blocking reagent), MBL, PN MTG-001

## Procedure for Whole Blood

1. Collect blood by venipuncture into a blood collection tube containing an appropriate anti-coagulant.
2. Add 10 µL of T-Select MHC Tetramer to each 12 x 75 mm test tube.
3. Add 200 µL of whole blood into each test tube.
4. Vortex gently.
5. Incubate for 30-60 minutes at 2-8°C or room temperature (15-25°C) protected from light.
6. Add any additional antibodies (e.g. anti-CD8) and vortex gently.
7. Incubate for 30 minutes at 2-8°C protected from light.
8. Lyse red blood cells using commercially available reagents.
9. Prepare samples according to description of the package insert.
10. Analyze prepared samples by flow cytometry. If necessary, store the samples at 2-8°C protected from light for a maximum of 24 hours prior to analysis.

## Procedure for Peripheral Blood Mononuclear Cells

1. Prepare peripheral blood mononuclear cells (PBMC) according to established procedures. Cells should be re-suspended at a concentration of  $2 \times 10^7$  cells/mL. 50 µL of sample is required for each T-Select MHC Tetramer determination.
2. Add 10 µL of Clear Back (human FcR blocking reagent, MBL, PN MTG-001) to each 12 x 75 mm test tube.
3. Add 50 µL PBMC into each test tube (e.g.  $1 \times 10^6$  cells per tube).

4. Incubate for 5 minutes at room temperature.
5. Add 10  $\mu$ L of T-Select MHC Tetramer and vortex gently.
6. Incubate for 30-60 minutes at 2-8°C or room temperature (15-25°C) protected from light.
7. Add any additional antibodies (e.g. anti-CD8) and vortex gently.
8. Incubate for 30 minutes at 2-8°C protected from light.
9. Add 3 mL of PBS or FCM buffer (2% FCS/0.09%  $\text{NaN}_3$ /PBS).
10. Centrifuge tubes at 400 x g for 5 minutes.
11. Aspirate or decant the supernatant.
12. Resuspend the pellet in 500  $\mu$ L of PBS with 0.5% formaldehyde.
13. Analyze prepared samples by flow cytometry. If necessary, store the samples at 2-8°C protected from light for a maximum of 24 hours prior to analysis.

#### Limitations

1. For optimal results with whole blood, retain specimens in blood collection tubes at room temperature, while rocking, prior to staining and analyzing. Refrigerated specimens may give aberrant results.
2. Recommended cell viability for venous blood specimens is > 90%.
3. Prolonged exposure of cells to lytic reagents may cause white blood cell destruction and loss of cells in the population of interest.
4. All red blood cells may not lyse under the following conditions: nucleated red blood cells, abnormal protein concentration or hemoglobinopathies. This may cause falsely decreased results due to unlysed red blood cells being counted as leukocytes.

#### Technical Hints

- A. If PBMC culture is needed, we recommend the use of heparin as an anti-coagulant.
- B. Clear Back reagent (human FcR blocking reagent) may effectively block non-specific binding caused by macrophages or endocytosis, resulting in clear staining when cells are stained with MHC Tetramer and antibodies. Please refer to the data sheet (MBL, PN MTG-001) for details.
- C. A Tetramer that is constructed with the same allele of interest and an irrelevant peptide may be used as a negative control.
- D. We recommend the use of anti-CD8 antibody, clone SFC121Thy2D3 (T8, MBL, PN 6603861), because some anti-CD8 antibodies inhibit Tetramer-specific binding to TCR.
- E. The use of CD45 antibody and gating of the lymphocyte population are recommended in order to reduce contamination of unlysed or nucleated red blood cells in the gate.
- F. Apoptotic, necrotic, and/or damaged cells are sources of interference in the analysis of viable

cells by flow cytometry. Cell viability should be determined by 7-aminoactinomycin D (7-AAD) staining; intact viable cells remain unstained (negative).

- G. Cells do not require fixation prior to analysis if the stained cells are analyzed by flow cytometry within several hours.

#### Related Products

##### HLA-E Tetramers

TS-ME01-1	HLA-E*01:03	HLA-A leader <sub>3-11</sub>	Tetramer-PE
TS-ME02-1	HLA-E*01:03	Negative	Tetramer-PE
TS-ME03-1	HLA-E*01:01	HLA-A leader <sub>3-11</sub>	Tetramer-PE
TS-ME04-1	HLA-E*01:01	Negative	Tetramer-PE

##### Others

4844	IMMUNOCYTO CD107a Detection Kit
8223	IMMUNOCYTO IFN- $\gamma$ ELISPOT Kit
AM-1005	IMMUNOCYTO Cytotoxicity Detection Kit
6603861	CD8-FITC (T8)
6607011	CD8-PC5 (T8)
6607102	CD8-PC7 (T8)
MTG-001	Clear Back (human FcR blocking reagent)
A07704	7-AAD Viability Dye

Please check our web site (<http://ruo.mbl.co.jp>) for up-to-date information on products and custom MHC Tetramers.

#### Experimental Data

PBMCs from healthy donors were collected from freshly isolated heparinized peripheral blood according to standard methods. HLA-E genotyping was not carried out.

##### Example 1:

Aliquots of the PBMCs ( $1 \times 10^6$  cells) were incubated with CD94 antibody (clone DX22, 20  $\mu$ g/mL) for 15 minutes at 4°C. Subsequently, cells were incubated with indicated HLA-E Tetramer, CD8 antibody and 7-AAD.

##### Example 2:

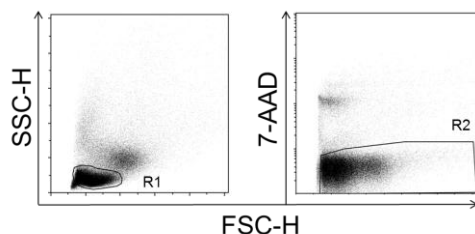
In the analysis of tetramer-binding to the TCR/CD3 complex, aliquots of the PBMCs ( $1 \times 10^6$  cells) were preincubated with CD3 antibody (clone OKT3, 100  $\mu$ g/mL) for 15 minutes at 4°C. Cells were washed and incubated with anti-mouse IgG (dilution 1:50, MBL, PN IM-0815) for 15 minutes at 4°C. Cells were washed and incubated with CD94 antibody for 15 minutes at 4°C. Subsequently, cells were incubated with indicated HLA-E Tetramer, CD8 antibody and 7-AAD.

##### Example 3:

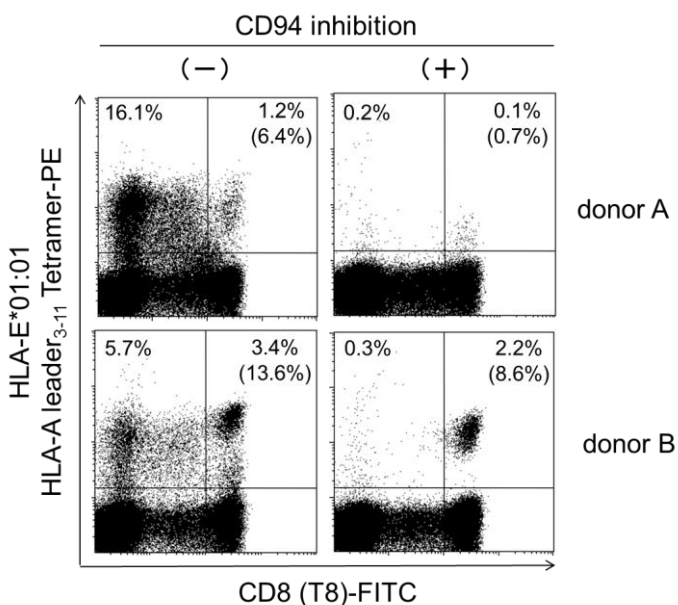
Aliquots of the PBMCs ( $1 \times 10^6$  cells) were incubated with indicated HLA-E Tetramer, CD56 antibody and 7-AAD.

## Results

The lymphocyte population was defined by an FSC/SSC gate (R1), and the viable cell population was defined by an FSC/7-AAD (R2). Data were analyzed by double gating on the lymphocyte and viable cell population (R1 and R2).

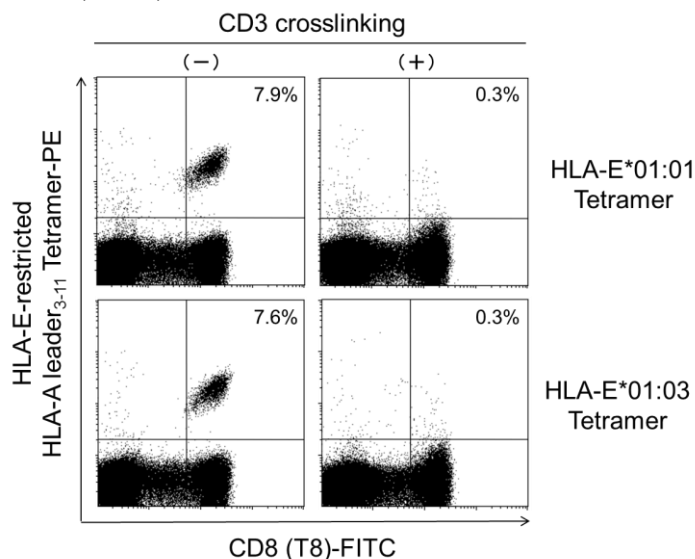


**Example 1:** Reactivity of the HLA-E tetramer with or without CD94 antibody.



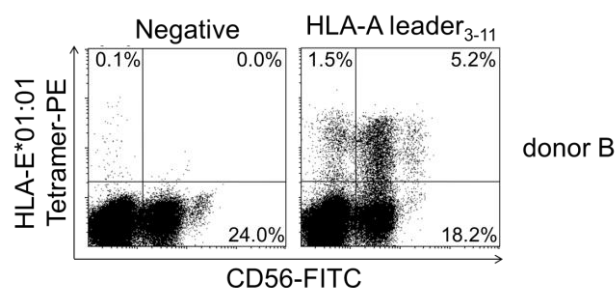
CD94 antibody-preincubation inhibited staining with HLA-E\*01:01 HLA-A leader<sub>3-11</sub> Tetramer via CD94/NKG2 receptor<sup>1</sup>. Numbers refer to percentage of the HLA-E tetramer-positive CD8<sup>+</sup> cells within total lymphocytes. Numbers in parentheses indicate the percentage of HLA-E tetramer-positive CD8<sup>+</sup> cells within total CD8<sup>+</sup> cells.

**Example 2:** Inhibition of the HLA-E tetramer-binding to T cells by preincubation with CD3 (OKT3) and CD94 antibodies.



Binding of HLA-E-restricted HLA-A leader<sub>3-11</sub> Tetramer to the TCR/CD3 complex was inhibited by preincubation with CD3 antibody followed by anti-mouse IgG in donor B<sup>11</sup>. In addition, the staining results of HLA-E\*01:01 and HLA-E\*01:03 Tetramer (MBL, PN TS-ME01-1) were almost the same. Numbers refer to percentages of the HLA-E tetramer-positive CD8<sup>+</sup> cells within total CD8<sup>+</sup> cells.

**Example 3:** Staining with HLA-E\*01:01 HLA-A leader<sub>3-11</sub> Tetramer or HLA-E\*01:01 Negative Tetramer.



Tetramer-positive CD56<sup>+</sup> cells were detected with the HLA-E\*01:01 HLA-A leader<sub>3-11</sub> Tetramer, whereas tetramer-positive CD56<sup>+</sup> cells were not detected with the HLA-E\*01:01 Negative Tetramer (MBL, PN TS-ME04-1). Numbers refer to percentages of cells within each quadrant against total lymphocytes.