**For Research Use Only.
Not for use in diagnostic procedures.**

T-Select MHC class I human Tetramer

HLA-E*01:03 Negative Tetramer-VMAPKTLVL-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 $CD8^+$ 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 $CD8^+$ 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 $CD8^+$ T cells. HLA-E is expressed in almost all tissues including lung, liver, skin and placental cells[3]. HLA-E expression is also detected in solid tumor (e.g., osteosarcoma and melanoma)[5]. HLA-E binds to TCR expressed on $CD8^+$ T cells, resulting in the T cells activation[4]. HLA-E is also known to bind CD94/NKG2 receptor expressed on NK cells and $CD8^+$ T cells[2]. CD94 can pair with several different isoforms of NKG2 to form receptors with potential to either inhibit (NKG2A, NKG2B) or promote (NKG2C) cellular activation.

This Tetramer reagent comprises human class I HLA-E*01:03 and epitope peptide derived from the HLA-A leader$_{3\text{-}11}$ containing lysine substitution at P5, and it can be used as a HLA-E*01:03 negative control Tetramer.

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[2]. It is reported that HLA-E*01:03 Negative Tetramer, generated with peptide from the HLA-A leader$_{3\text{-}11}$ containing lysine substitution at P5, does not bind NK cells[1].

HLA Restriction: HLA-E*01:03

Origin and Sequence of CTL Epitope

HLA-A*02, A*24 leader R5K (3-11 aa, VMAPKTLVL).

References for This Product

1) Miller JD, *et al*. *J Immunol* **171**: 1369-1375 (2003)
2) Braud VM, *et al. Nature* **391**: 795-799 (1998)
3) Lee N, *et al. PNAS* **95**: 5199-5204 (1998)
4) Pietra G, *et al*. *PNAS* **100**: 10896-10901 (2003)
5) Monaco EL, *et al. Neoplasia* **13**: 822-830 (2011)

Reagents

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_3.

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.

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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
- MHC Tetramer Lyse Reagent, MBLI, PN T08002
- MHC Tetramer Fixative Reagent, MBLI, PN T08003
- 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 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 2 mL of Lyse Reagent supplemented with 50 μL Fixative Reagent per tube.
9. Vortex for 5 seconds immediately after the addition of the Lyse/Fixative solution.
10. Incubate for a minimum of 10 minutes at room temperature protected from light.
11. Centrifuge tubes at 150 x g for 5 minutes.
12. Aspirate or decant the supernatant.
13. Add 3 mL of PBS and centrifuge tubes at 150 x g for 5 minutes.
14. Aspirate or decant the supernatant.
15. Resuspend the pellet in 500 μL of PBS with 0.1% formaldehyde. (12.5 μL Fixative Reagent/1 mL PBS).
16. Store prepared samples at 2-8°C protected from light for a minimum of 1 hour (maximum 24 hours) prior to analysis by flow cytometry.

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 x 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 x 10^6 cells per tube).
4. Incubate for 5 minutes at room temperature.
5. Add 10 μL of T-Select MHC Tetramer and vortex gently.
6. Incubate for 30-60 minutes at 2-8°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% 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 μL of PBS with 0.5% formaldehyde. (62.5 μL Fixative Reagent/1 mL PBS).
13. Store prepared samples at 2-8°C protected from light for a minimum of 1 hour (maximum 24 hours) prior to analysis by flow cytometry.

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 SFCI21Thy2D3 (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$_{3\text{-}11}$ Tetramer-PE
TS-ME02-1 HLA-E*01:03 Negative Tetramer-PE

Others

4844	IMMUNOCYTO CD107a Detection Kit
8223	IMMUNOCYTO IFN-γ ELISPOT Kit
AM-1005	IMMUNOCYTO Cytotoxity Detection Kit
MTG-001	Clear Back (human FcR blocking reagent)
A07704	7-AAD Viability Dye

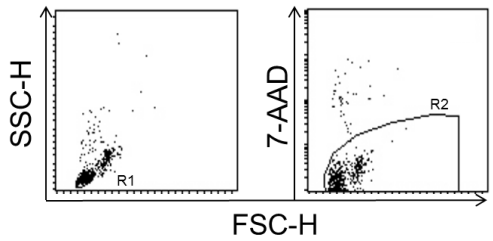
Please check our web site (http://ruo.mbl.co.jp) for up-to-date information on products and custom MHC Tetramers.

Experimental Data

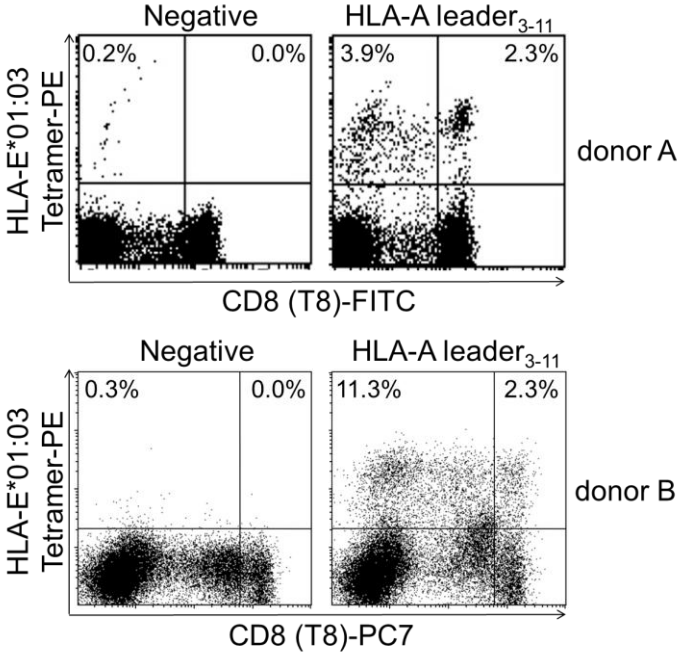
PBMCs from healthy donors were collected from freshly isolated heparinized peripheral blood according to standard methods. Aliquots of the PBMCs (1 x 10^6 cells) were incubated with indicated HLA-E Tetramer, CD8 or CD56 antibody and 7-AAD. HLA-E genotyping was not carried out.

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).

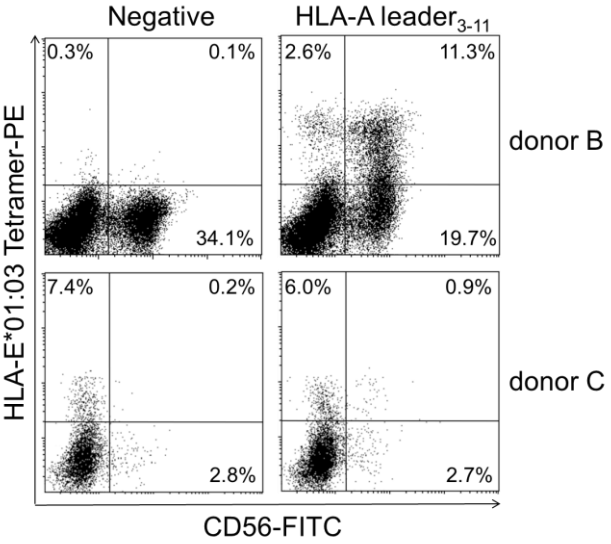


Example 1: Staining with HLA-E*01:03 HLA-A leader$_{3-11}$ Tetramer or HLA-E*01:03 Negative Tetramer.



Tetramer-positive CD8$^+$ cells were detected with the HLA-E*01:03 HLA-A leader$_{3-11}$ Tetramer, whereas tetramer-positive cells were not detected with the HLA-E*01:03 Negative Tetramer. Numbers refer to percentages of tetramer-positive cells within total lymphocytes.

Example 2: Staining with HLA-E*01:03 HLA-A leader$_{3-11}$ Tetramer or HLA-E*01:03 Negative Tetramer.



Tetramer-positive CD56$^+$ cells were detected with the HLA-E*01:03 HLA-A leader$_{3-11}$ Tetramer, whereas tetramer-positive CD56$^+$ cells were not detected with the HLA-E*01:03 Negative Tetramer. Numbers refer to percentages of tetramer-positive cells within total lymphocytes.