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

T-Select MHC Tetramer

HLA-A*02:01 $WT1_{37-45}$ Tetramer -VLDFAPPGA (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 with 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.

This Tetramer reagent comprises human class I HLA-A*02:01 and WT1-derived epitope peptide, $WT1_{37-45}$, and it can detect HLA-A*02:01-restricted $WT1_{37-45}$-specific $CD8^+$ T cells by flow cytometry.

Wilms' tumor gene 1 (WT1) is a zinc finger transcription factor with limited expression in normal adult tissues, but is overexpressed in the majority of leukemia and various types of solid tumors. In 2009, WT1 was ranked first in a list of 75 representative cancer antigens in a National Cancer Institute prioritization project. It has been shown that WT1-specific CTLs kill tumor cells with high WT1 expression, but not normal cells. Many clinical trials of cancer immunotherapy targeting the WT1 have been carried out around the world.

A Tetramer, which is constructed with the same allele (HLA-A*02:01) of interest and an irrelevant peptide, may be used as a negative control Tetramer.

HLA Restriction

HLA-A*02:01

Origin and Sequence of CTL Epitope

WT1 (37-45 aa, VLDFAPPGA)

References for WT1 and This Epitope

1) Call KM, *et al. Cell* **60**: 509-520 (1990)
2) Gessler M, *et al. Nature* **343**: 774-778 (1990)
3) Smithgall M, *et al*. *Blood* (ASH Annual Meeting Abstracts). **98**: Abstract 121a (2001)
4) Rezvani K, *et al. Clin Cancer Res* **11**: 8799-8807 (2005).
5) Chaise C, *et al*. *Blood* **112**: 2956-2964 (2008)

Conjugates

TS-M140-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M140-2
Streptavidin-Allophycocyanin (SA-APC)
Excites at 633-635 nm
Emits at 660-680 nm

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_3.

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), or light blue (SA-APC).

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 × 75 mm polypropylene test tubes
- Transfer pipettes
- Pipettors and disposable pipette tips
- Vortex mixer
- Centrifuge capable of 150 × g or 400 × g
- Aspirator
- PBS
- Red blood cell lysis reagent
- Anti-CD8-FITC (T8), Beckman Coulter, Inc., PN 6603861
- Anti-CD8-PC5 (T8), Beckman Coulter, Inc., PN 6607011
- 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 × 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. 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 2×10^7 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 × 75 mm test tube.
3. Add 50 μL PBMC into each test tube (e.g. 1×10^6 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 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% NaN_3/PBS).
10. Centrifuge tubes at 400 × g for 5 minutes.
11. Aspirate or decant the supernatant.
12. Resuspend the pellet in 500 μL of PBS with 0.5% formaldehyde.
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, Beckman Coulter, Inc.), 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

WT1 Tetramers and Peptide

TS-M140-1	HLA-A*02:01 $WT1_{37-45}$ Tetramer-VLDFAPPGA-PE
TS-M140-P	HLA-A*02:01 $WT1_{37-45}$ peptide
TS-M016-1	HLA-A*02:01 $WT1_{126-134}$ Tetramer-RMFPNAPYL-PE
TS-M014-1	HLA-A*24:02 modified WT1 Tetramer-CYTWNQMNL-PE
TS-M504-1	H-$2D^b$ $WT1_{126-134}$ Tetramer-RMFPNAPYL-PE

Human Tetramers

Cancer

TS-M141-1	HLA-A*24:02 ACC-1 Tetramer-DYLQYVLQI-PE
TS-M137-1	HLA-A*01:01 AIM-2 Tetramer-RSDSGQQARY-PE
TS-M112-1	HLA-A*24:02 $CA9_{219-227}$ Tetramer-EYRALQLHL-PE
TS-M103-1	HLA-A*02:01 CEA Tetramer-YLSGANLNL-PE
TS-M080-1	HLA-A*02:01 CEA (N6D) Tetramer-YLSGADLNL-PE
TS-M101-1	HLA-A*02:01 CD33 Tetramer-AIISGDSPV-PE
TS-M102-1	HLA-A*02:01 CD33 A65Y Tetramer-YIISGDSPV-PE
TS-M084-1	HLA-A*02:01 EphA2 Tetramer-TLADFDPRV-PE
TS-0014-1C	HLA-A*02:01 gp100 (wild) Tetramer-ITDQVPFSV-PE
TS-0013-1C	HLA-A*02:01 gp100 (mutant) Tetramer-IMDQVPFSV-PE
TS-0035-1C	HLA-A*02:01 $gp100_{154-162}$ Tetramer-KTWGQYWQV-PE
TS-M082-1	HLA-A*02:01 gp100 Tetramer-YLEPGPVTA-PE
TS-M089-1	HLA-A*24:02 gp100-intron 4 Tetramer-VYFFLPDHL-PE
TS-0016-1	HLA-A*02:01 Her-2/neu Tetramer-RLLQETELV-PE
TS-0015-1C	HLA-A*02:01 Her-2/neu E75 Tetramer-KIFGSLAFL-PE
TS-M083-1	HLA-A*02:01 HM1.24 Tetramer-KLQDASAEV-PE
TS-M010-1	HLA-A*24:02 hTERT Tetramer-VYGFVRACL-PE
TS-M115-1	HLA-A*02:01 hTERT Tetramer-ILAKFLHWL-PE
TS-M086-1	HLA-A*02:01 IDO Tetramer-ALLEIASCL-PE
TS-M070-1	HLA-A*02:01 MAGE-A1 Tetramer-KVLEYVIKV-PE
TS-M071-1	HLA-B*07:02 MAGE-A1 Tetramer-RVRFFFPSL-PE
TS-M072-1	HLA-A*02:01 MAGE-A2 Tetramer-YLQLVFGIEV-PE
TS-M073-1	HLA-A*24:02 MAGE-A2 Tetramer-EYLQLVFGI-PE
TS-M075-1	HLA-A*02:01 $MAGE-A3_{112-120}$ Tetramer-KVAELVHFL-PE
TS-M076-1	HLA-A*02:01 $MAGE-A3_{271-279}$ Tetramer-FLWGPRALV-PE
TS-M077-1	HLA-A*24:02 MAGE-A3 Tetramer-IMPKAGLLI-PE
TS-M078-1	HLA-A*02:01 MAGE-A10 Tetramer-GLYDGMEHL-PE
TS-M138-1	HLA-A*02:01 MAGE-C1 Tetramer-ILFGISLREV-PE
TS-0009-1C	HLA-A*02:01 Mart-1 Tetramer-ELAGIGILTV-PE
TS-M091-1	HLA-A*24:02 MCPyV large TAg Tetramer-EWWRSGGFSF-PE
TS-M088-1	HLA-A*02:01 MUC1 Tetramer-LLLLTVLTV-PE
TS-M011-1	HLA-A*02:01 NY-ESO-1 Tetramer-SLLMWITQC-PE
TS-M105-1	HLA-A*02:01 NY-ESO-1 C9V Tetramer-SLLMWITQV-PE
TS-M109-1	HLA-B*07:02 P2X5 Tetramer-TPNQRQNVC-PE
TS-M081-1	HLA-A*02:01 p53 Tetramer-LLGRNSFEV-PE
TS-M107-1	HLA-A*02:01 $PAP_{299-307}$ Tetramer-ALDVYNGLL-PE
TS-M136-1	HLA-A*24:02 PBF A24.2 Tetramer-AYRPVSRNI-PE
TS-M117-1	HLA-A*02:01 $PRAME_{100-108}$ Tetramer-VLDGLDVLL-PE
TS-M119-1	HLA-A*02:01 $PRAME_{142-151}$ Tetramer-SLYSFPEPEA-PE
TS-M116-1	HLA-A*02:01 $PRAME_{300-309}$ Tetramer-ALYVDSLFFL-PE
TS-M118-1	HLA-A*02:01 $PRAME_{425-433}$ Tetramer-SLLQHLIGL-PE
TS-M120-1	HLA-A*02:01 $PSA_{141-150}$ Tetramer-FLTPKKLQCV-PE
TS-0017-1	HLA-A*02:01 PR-1 Tetramer-VLQELNVTV-PE
TS-M087-1	HLA-A*02:01 PSA Tetramer-KLQCVDLHV-PE
TS-M104-1	HLA-A*02:01 RHAMM Tetramer-ILSLELMKL-PE
TS-M095-1	HLA-A*02:01 PP2A Tetramer-SLLPAIVEL-PE
TS-M079-1	HLA-A*02:01 SSX-2 Tetramer-KASEKIFYV-PE
TS-M025-1	HLA-A*24:02 survivin-2B Tetramer-AYACNTSTL-PE
TS-M085-1	HLA-A*02:01 Survivin (T2M) Tetramer-LMLGEFLKL-PE
TS-0019-1C	HLA-A*02:01 Tyrosinase Tetramer-YMDGTMSQV-PE
TS-M090-1	HLA-A*24:02 Tyrosinase Tetramer-AFLPWHRLF-PE

Kits

4844	IMMUNOCYTO CD107a Detection Kit
8223	IMMUNOCYTO IFN-γ ELISPOT Kit
AM-1005	IMMUNOCYTO Cytotoxicity Detection Kit

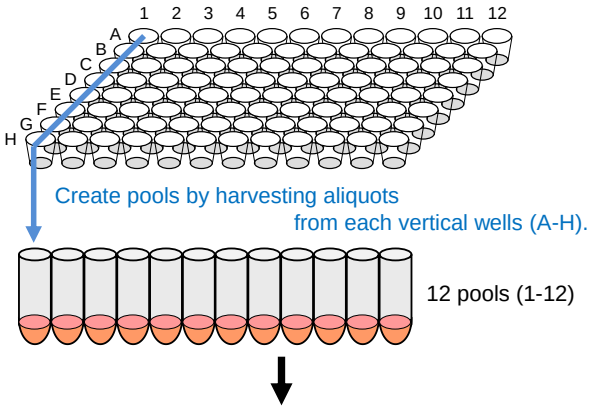
Please check our web site (http://ruo.mbl.co.jp) for up-to-date information on products and custom MHC Tetramers.

Experimental Data

WT1$_{37-45}$-specific CTLs were induced from commercial cryopreserved HLA-A*02:01-positive PBMCs by the mixed lymphocyte peptide culture under limiting dilution conditions (LD/MLPC), and were stained with MHC Tetramer.

LD/MLPC (Mixed-Lymphocyte-Peptide Cultures under limiting dilution conditions)
modified by MBL

Culture PBMC with antigenic peptide in 96-well plate for 2 weeks.
(RPMI1640/5% autoplasma/antigenic peptide/IL-2 [50 U/mL, day 2])



T-Select MHC Tetramer stain

The thawed and washed PBMCs were suspended in RPMI1640 supplemented with 10% human AB serum (HS), and incubated over night at 37°C. The PBMCs were suspended at the concentration of 3 × 10^6 cells/mL with RPMI1640 with 10% HS and stimulated with WT1$_{37-45}$ peptide (10 μg/mL of VLDFAPPGA, MBL, PN TS-M140-P). Then, the peptide-pulsed PBMCs were seeded into wells at the concentration of 3 × 10^5 cells/100 μL/well into round-bottom 96-well plates and incubated. After 24 hours, 100 μL of fresh RPMI1640 containing 10% HS and 100 U/mL interleukin-2 (IL-2) was added to each well (final concentration: 50 U/mL IL-2). Thereafter, every 2 to 3 days, half of the medium was replaced with fresh medium containing 10% HS and IL-2 (50 U/ml).

On day 13 to 14, these cultured cells were stained with specific MHC Tetramer and CD8 antibody as follows: First, an aliquot of cells from each of 8 vertical wells of a 96-well plate were collected to make 12 pooled samples. These samples were stained separately with MHC Tetramer. Second, to identify the MHC Tetramer-positive well, the cells in the individual wells within the positive pool were respectively stained with MHC Tetramer.

Staining procedure

1. Cells were once washed and re-suspended in FCM buffer [2% FCS/0.05% NaN_3/PBS]. Optimally, 50 μL of sample containing 1 × 10^6 cells (2 × 10^7 cells/mL) is required for MHC Tetramer staining.
2. 10 μL of Clear Back (human FcR blocking reagent, MBL, PN MTG-001) was added and incubated for 5 minutes at room temperature.
3. 10 μL of MHC Tetramer (HLA-A*02:01 WT1$_{37-45}$ Tetramer; MBL, PN TS-M140-1) was added and incubated for 20 minutes at 4°C protected from light.
4. 10 μL of CD8 (T8)-FITC (MBL, PN 6603861) was added and incubated for 20 minutes at 4°C protected from light.
5. 1 mL of FCM buffer was added and centrifuged at 400 × g for 5 minutes.
6. The supernatant was discarded carefully and the pellet was re-suspended in 400 μL of FCM buffer.
7. 5 μL of 7-AAD Viability Dye (MBL, PN A07704) was added and vortexed gently, and then the sample was analyzed by flow cytometry.

Result

The lymphocyte population was defined by an FSC/SSC gate, and the viable cell population was defined by an FSC/7-AAD. Data were analyzed by double gating on the lymphocyte and viable cell population. Numbers in the top right quadrants represent the percentage of MHC Tetramer-positive cells in the total CD8$^+$ cells.

The WT1$_{37-45}$-specific CTLs were induced from PBMCs at a rate of 18 (donor A), 3.69 (donor B) and 1.2 wells (donor C) per 96-well plate.