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

T-Select MHC Tetramer

HLA-A*24:02 PBF A24.2 Tetramer -AYRPVSRNI (50 tests)

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MBL manufactures and distributes these products under license from Beckman Coulter, Inc.. These T-Select MHC Tetramers use patented technology (Japanese patent No. P4484707) of Sapporo Medical University.

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*24:02 and epitope peptide derived from PBF, and it can detect HLA-A*24:02-restricted PBF A24.2-specific $CD8^+$ T cells by flow cytometry.

Papillomavirus binding factor (PBF) was first identified as a transcription factor regulating the promoter activity of human papillomavirus.

Dr. Noriyuki Sato and his colleagues at Sapporo Medical University demonstrated that PBF was an osteosarcoma-associated antigen and 92% of osteosarcoma tissues expressed PBF in the nucleus. Moreover, PBF-positive osteosarcoma had a significantly poorer prognosis than that with negative expression of PBF. They identified some CTL epitope peptides (restricted by HLA-A2, A24, and B55) derived from PBF and have conducted clinical trials to develop peptide-based immunotherapy.

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)

HLA Restriction

HLA-A*24:02

Origin and Sequence of CTL Epitope

PBF A24.2 (145-153 aa, AYRPVSRNI)

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.

Conjugates

TS-M136-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M136-2
Streptavidin-Allophycocyanin (SA-APC)
Excites at 633-635 nm
Emits at 660-680 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.

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp

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.

References for These Products

1) Tsukahara T, *et al*. *Cancer Res* **64**: 5442-5448 (2004)
2) Tsukahara T, *et al*. *Cancer Sci* **99**: 368-375 (2008)
3) Yabe H, *et al*. *Oncol Rep* **19**: 129-134 (2008)
4) Tsukahara T, *et al*. *J Transl Med* **7**: 44 (2009)

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
- Anti-CD8-PC5, 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 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. 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 x 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 x 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

T-Select Human Tetramers

Cancer

TS-M014-1 HLA-A*24:02 WT1 (mutant) Tetramer-CYTWNQMNL-PE
TS-M014-2 HLA-A*24:02 WT1 (mutant) Tetramer-CYTWNQMNL-APC
TS-M016-1 HLA-A*02:01 WT1 Tetramer-RMFPNAPYL-PE
TS-M016-2 HLA-A*02:01 WT1 Tetramer-RMFPNAPYL-APC
TS-M010-1 HLA-A*24:02 hTERT Tetramer-VYGFVRACL-PE
TS-M115-1 HLA-A*02:01 hTERT Tetramer-ILAKFLHWL-PE
TS-M115-2 HLA-A*02:01 hTERT Tetramer-ILAKFLHWL-APC
TS-M011-1 HLA-A*02:01 NY-ESO-1 Tetramer-SLLMWITQC-PE
TS-M011-2 HLA-A*02:01 NY-ESO-1 Tetramer-SLLMWITQC-APC
TS-M105-1 HLA-A*02:01 NY-ESO-1 C9V Tetramer-SLLMWITQV-PE
TS-M105-2 HLA-A*02:01 NY-ESO-1 C9V Tetramer-SLLMWITQV-APC
TS-M025-1 HLA-A*24:02 survivin-2B Tetramer-AYACNTSTL-PE
TS-0009-1C HLA-A*02:01 Mart-1 Tetramer-ELAGIGILTV-PE
TS-0013-1C HLA-A*02:01 gp100 Tetramer-IMDQVPFSV-PE
TS-0014-1C HLA-A*02:01 gp100 Tetramer-ITDQVPFSV-PE
TS-0015-1C HLA-A*02:01 Her-2/neu Tetramer-KIFGSLAFL-PE
TS-0016-1 HLA-A*02:01 Her-2/neu Tetramer-RLLQETELV-PE
TS-0017-1 HLA-A*02:01 PR-1 Tetramer-VLQELNVTV-PE
TS-0019-1C HLA-A*02:01 Tyrosinase Tetramer-YMDGTMSQV-PE
TS-M114-1 HLA-A*01:01 MAGE-A1 Tetramer-EADPTGHSY-PE
TS-M114-2 HLA-A*01:01 MAGE-A1 Tetramer-EADPTGHSY-APC
TS-M101-1 HLA-A*02:01 CD33 Tetramer-AIISGDSPV-PE
TS-M101-2 HLA-A*02:01 CD33 Tetramer-AIISGDSPV-APC
TS-M102-1 HLA-A*02:01 CD33 A65Y Tetramer-YIISGDSPV-PE
TS-M102-2 HLA-A*02:01 CD33 A65Y Tetramer-YIISGDSPV-APC
TS-M103-1 HLA-A*02:01 CEA Tetramer-YLSGANLNL-PE
TS-M103-2 HLA-A*02:01 CEA Tetramer-YLSGANLNL-APC
TS-M104-1 HLA-A*02:01 RHAMM Tetramer-ILSLELMKL-PE
TS-M104-2 HLA-A*02:01 RHAMM Tetramer-ILSLELMKL-APC
TS-M116-1 HLA-A*02:01 $PRAME_{300-309}$ Tetramer-ALYVDSLFFL-PE
TS-M116-2 HLA-A*02:01 $PRAME_{300-309}$ Tetramer-ALYVDSLFFL-APC
TS-M117-1 HLA-A*02:01 $PRAME_{100-108}$ Tetramer-VLDGLDVLL-PE
TS-M117-2 HLA-A*02:01 $PRAME_{100-108}$ Tetramer-VLDGLDVLL-APC
TS-M118-1 HLA-A*02:01 $PRAME_{425-433}$ Tetramer-SLLQHLIGL-PE
TS-M118-2 HLA-A*02:01 $PRAME_{425-433}$ Tetramer-SLLQHLIGL-APC
TS-M119-1 HLA-A*02:01 $PRAME_{142-151}$ Tetramer-SLYSFPEPEA-PE
TS-M119-2 HLA-A*02:01 $PRAME_{142-151}$ Tetramer-SLYSFPEPEA-APC
TS-M120-1 HLA-A*02:01 $PSA_{141-150}$ Tetramer-FLTPKKLQCV-PE
TS-M120-2 HLA-A*02:01 $PSA_{141-150}$ Tetramer-FLTPKKLQCV-APC
TS-M136-1 HLA-A*24:02 PBF A24.2 Tetramer-AYRPVSRNI-PE
TS-M136-2 HLA-A*24:02 PBF A24.2 Tetramer-AYRPVSRNI-APC

Control

TS-M007-1 HLA-A*24:02 Negative Tetramer-RYLRDQQLL-PE
TS-M007-2 HLA-A*24:02 Negative Tetramer-RYLRDQQLL-APC
TS-M007-3 HLA-A*24:02 Negative Tetramer-RYLRDQQLL-FITC
TS-0029-1C HLA-A*02:01 Negative Tetramer-PE
TS-0029-2C HLA-A*02:01 Negative Tetramer-APC

T-Select Peptides

TS-M007-P HLA-A*24:02 HIV env gp160 peptide
TS-M011-P HLA-A*02:01 NY-ESO-1 peptide
TS-M025-P HLA-A*24:02 survivin-2B peptide
TS-M026-P HLA-A*02:01 MPT51 peptide
TS-0009-P HLA-A*02:01 Mart-1 peptide
TS-M136-P HLA-A*24:02 PBF A24.2 peptide

Others

4844	IMMUNOCYTO CD107a Detection Kit
8223	IMMUNOCYTO IFN-γ ELISPOT Kit
AM-1005	IMMUNOCYTO Cytotoxity Detection Kit
6603861	CD8-FITC (T8)
6607011	CD8-PC5 (T8)
A07704	7-AAD Viability Dye
MTG-001	Clear Back (Human FcR blocking reagent)

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

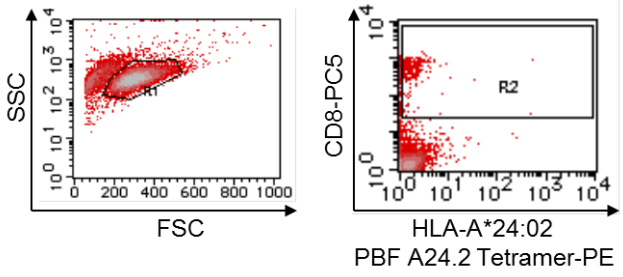
Experimental Data

These data were kindly provided by Dr. Tomohide Tsukahara and Dr. Toshihiko Torigoe, Department of Pathology, Sapporo Medical University.

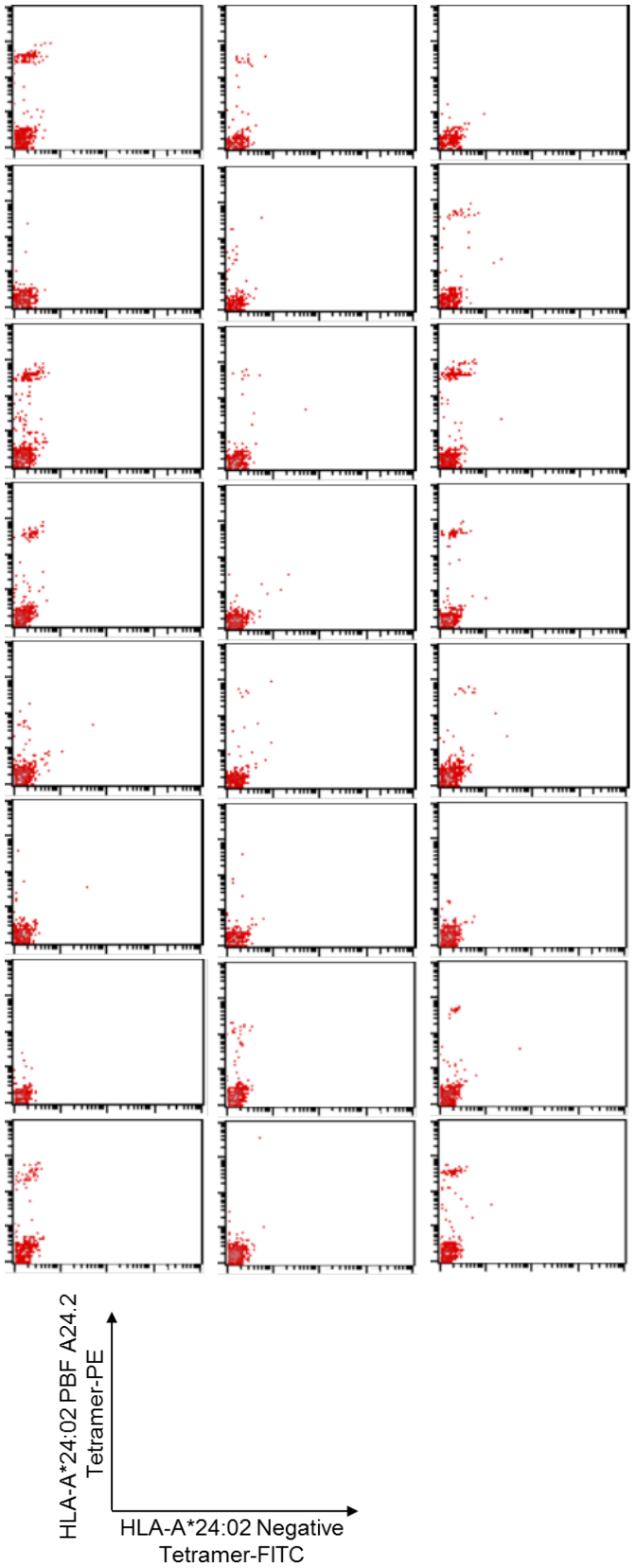
PBMCs isolated from an HLA-A24-positive patient with osteosarcoma were subjected to mixed lymphocyte peptide culture under limiting dilution conditions (LD/MLPC). PBMCs were suspended in AIM-V supplemented with 1% human serum (HS) and incubated for 60 min at room temperature with PBF A24.2 peptide (50 μg/mL of AYRPVSRNI, MBL, PN TS-M136-P). Peptide-pulsed PBMC were seeded into 62 wells at the concentration of 2×10^5 cells/200 μL/well into round-bottom 96-microwell plates in AIM-V with 10% HS, IL-2 (20 U/mL) and IL-7 (10 ng/mL), and incubated. On day 7, half of the medium was replaced by fresh AIM-V containing IL-2, IL-7 and the same peptide. The cell cultures were maintained by adding fresh AIM-V containing IL-2. On day 14, these cultured cells were subjected to tetramer-based frequency analysis.

From each microwell containing 200 μL of the microculture pool, 100 μL was transferred to a V-bottom microwell and washed. To the spin-down pellets, an HLA-A*24:02 Negative Tetramer-FITC (here termed the control tetramer, MBL, PN TS-M007-3) and an HLA-A*24:02 PBF A24.2 Tetramer-PE (MBL, PN TS-M136-1) were added in combination and incubated for 15 min at room temperature. Then a PC5-conjugated anti-CD8 antibody was added and incubated for another 15 min. The cells were washed in PBS twice, fixed with 0.5% formaldehyde, and analyzed by flow cytometry.

Result:



Data were analyzed by double gating on the living lymphocyte (R1) and $CD8^+$ cells (R2).



To confirm specificity of MHC Tetramer staining, cells were stained with both specific and negative control MHC Tetramer (MBL, PN TS-M007-3) containing the peptide RYLRDQQLL, derived from the human immunodeficiency virus envelope (HIV env) protein.