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

T-Select MHC Class I Human Tetramer

Allele and Peptide Specificity

The T-Select MHC Class I Human Tetramers recognize human $CD8^+$ T cells which are specific for a particular peptide in combination with the HLA allele. The HLA molecule in this reagent has been modified to minimize CD8 mediated binding[1].

Background

T lymphocytes play a central role in immune system function. 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 class I MHC Tetramers which are composed of a complex of four HLA class I molecules each bound to the specific peptide[2), 3)] 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 all specific $CD8^+$ T cells regardless of functional status. Measurements may be performed in whole blood or isolated lymphocyte/mononuclear cell preparations. 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.

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)

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

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

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

- Streptavidin-Fluorescein Isothiocyanate (SA-FITC)
  Excites at 465-495 nm
  Emits at 515-555 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.
If the expiration date is not indicated, T-Select MHC Tetramers are stable for 90 days from the date of purchase. Stability data are not available for custom T-Select MHC Tetramers.

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), light blue (SA-APC), or light yellow liquid (SA-FITC).

Reagent Preparation

No preparation is necessary. These T-Select MHC Tetramer reagents are used directly from the vial after a brief vortex on low setting.

Usage

This reagent is for use with standard flow cytometry methodologies.

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 pipet 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 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 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 performed, we recommend the use of heparin as an anti-coagulant.
B. In an experiment where cells are stained with T-Select MHC Tetramer and antibodies, Clear Back (human FcR blocking reagent) may effectively block non-specific binding caused by macrophages or endocytosis, resulting in clear staining. Please refer to the data sheet (MBL, PN MTG-001) for details.
C. A Tetramer, which is constructed with the same allele of interest and an irrelevant peptide, may also 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. To reduce contamination of unlysed or nucleated red blood cells in the gate, we recommend the use

of CD45 antibody and gating of the lymphocyte population.

F. Apoptotic, necrotic, and/or damaged cells are sources of interference in the analysis of viable cells by flow cytometry. Non-viable cells should be evaluated and discriminated following 7-AAD-positive labeling when viable cells remain unstained (negative).

G. The cells do not need to be fixed before analysis if stained cells are analyzed by flow cytometry within several hours.

Selected References

1) Bodinier M, Peyrat M-A, Tournay C, Davodeau F, Romagne F, Bonneville M, and Lang F, 2000. Efficient Detection and Immunomagnetic Sorting of Specific T Cells Using Multimers of MHC Class I and Peptide with Reduced CD8 Binding. Nat. Med., 6:707-710.
2) Altman JD, Moss PH, Goulder PJR, Barouch DH, McHeyzer W, Bell JI, McMichael AJ, and Davis MM. 1996. Phenotypic Analysis of Antigen-Specific T Lymphocytes. Science 274:94-96.
3) McMichael AJ, and O 'Callaghan CA. 1998. A New Look at T Cells. J. Exp. Med. 187:1367-1371.

Related Products

T-Select Human Tetramers

Cancer

TS-M014-1	HLA-A*24:02 WT1 (mutant) Tetramer-CYTWNQMNL-PE
TS-M016-1	HLA-A*02:01 WT1 Tetramer-RMFPNAPYL-PE
TS-M010-1	HLA-A*24:02 hTERT Tetramer-VYGFVRACL-PE
TS-M115-1	HLA-A*02:01 hTERT Tetramer-ILAKFLHWL-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-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-M101-1	HLA-A*02:01 CD33 Tetramer-AIISGDSPV-PE
TS-M102-1	HLA-A*02:01 CD33 A65Y Tetramer-YIISGDSPV-PE
TS-M103-1	HLA-A*02:01 CEA Tetramer-YLSGANLNL-PE
TS-M104-1	HLA-A*02:01 RHAMM Tetramer-ILSLELMKL-PE
TS-M116-1	HLA-A*02:01 $PRAME_{300-309}$ Tetramer-ALYVDSLFFL-PE
TS-M117-1	HLA-A*02:01 $PRAME_{100-108}$ Tetramer-VLDGLDVLL-PE
TS-M118-1	HLA-A*02:01 $PRAME_{425-433}$ Tetramer-SLLQHLIGL-PE
TS-M119-1	HLA-A*02:01 $PRAME_{142-151}$ Tetramer-SLYSFPEPEA-PE
TS-M120-1	HLA-A*02:01 $PSA_{141-150}$ Tetramer-FLTPKKLQCV-PE

CMV

TS-M012-1	HLA-A*11:01 CMV pp65 Tetramer-ATVQGQNLK-PE
TS-0010-1C	HLA-A*02:01 CMV pp65 Tetramer-NLVPMVATV-PE
TS-0020-1C	HLA-A*24:02 CMV pp65 Tetramer-QYDPVAALF-PE
TS-M013-1	HLA-B*15:01 CMV pp65 Tetramer-KMQVIGDQY-PE
TS-0027-1C	HLA-B*35:01 CMV pp65 Tetramer-IPSINVHHY-PE
TS-0025-1C	HLA-B*07:02 CMV pp65 Tetramer-TPRVTGGGAM-PE
TS-M099-1	HLA-B*07:02 CMV pp65 Tetramer-RPHERNGFTVL-PE
TS-0024-1C	HLA-A*01:01 CMV pp50 Tetramer-VTEHDTLLY-PE
TS-M057-1	HLA-A*02:01 CMV IE1 Tetramer-VLEETSVML-PE
TS-M100-1	HLA-A*03:01 CMV IE1 Tetramer-KLGGALQAK-PE
TS-0026-1C	HLA-B*08:01 CMV IE1 Tetramer-ELRRKMMYM-PE

HTLV-1

TS-M017-1	HLA-A*02:01 HTLV-1 Tax_{11-19} Tetramer-PE
TS-M019-1	HLA-A*02:01 HTLV-1 $Tax_{178-186}$ Tetramer-PE
TS-M020-1	HLA-A*24:02 HTLV-1 Tax_{12-20} Tetramer-PE
TS-M021-1	HLA-A*24:02 HTLV-1 $Tax_{187-195}$ Tetramer-PE
TS-M018-1	HLA-A*24:02 HTLV-1 $Tax_{301-309}$ Tetramer-PE
TS-M022-1	HLA-A*24:02 HTLV-1 Env_{11-19} Tetramer-PE
TS-M023-1	HLA-A*11:01 HTLV-1 Tax_{88-96} Tetramer-PE
TS-M024-1	HLA-A*11:01 HTLV-1 $Tax_{272-280}$ Tetramer-PE

EBV

TS-0011-1C	HLA-A*02:01 EBV BMLF1 Tetramer-GLCTLVAML-PE
TS-M003-1	HLA-A*24:02 EBV BMLF1 Tetramer-DYNFVKQLF-PE
TS-M006-1	HLA-A*02:01 EBV LMP1 Tetramer-YLQQNWWTL-PE
TS-M030-1	HLA-A*02:01 EBV LMP2 Tetramer-TVCGGIMFL-PE
TS-M031-1	HLA-A*02:01 EBV LMP2 Tetramer-LLWTLVVLL-PE

TS-M069-1 HLA-A*02:01 EBV LMP2 Tetramer-FLYALALLL-PE
TS-M032-1 HLA-A*02:01 EBV LMP2 Tetramer-CLGGLLTMV-PE
TS-M034-1 HLA-A*24:02 EBV LMP2 Tetramer-PYLFWLAAI-PE
TS-M001-1 HLA-A*24:02 EBV LMP2 Tetramer-IYVLVMLVL-PE
TS-M035-1 HLA-A*24:02 EBV LMP2 Tetramer-TYGPVFMSL-PE
TS-M111-1 HLA-A*11:01 EBV LMP2 Tetramer-SSCSSCPLSK-PE
TS-M135-1 HLA-A*11:01 EBV LMP2 S9T Tetramer-SSCSSCPLTK-PE
TS-M038-1 HLA-B*35:01 EBV LMP2 Tetramer-MGSLEMVPM-PE
TS-M002-1 HLA-A*24:02 EBV BRLF1 Tetramer-TYPVLEEMF-PE
TS-M004-1 HLA-A*24:02 EBV EBNA3A Tetramer-RYSIFFDYM-PE
TS-M005-1 HLA-A*24:02 EBV EBNA3B Tetramer-TYSAGIVQI-PE
TS-M028-1 HLA-A*11:01 EBV EBNA3B$_{399-408}$ Tetramer-PE
TS-M029-1 HLA-A*11:01 EBV EBNA3B$_{416-424}$ Tetramer-PE
TS-M036-1 HLA-A*08:01 EBV BZLF1 Tetramer-RAKFKQLL-PE
TS-M037-1 HLA-B*35:01 EBV BZLF1 Tetramer-EPLPQGQLTAY-PE
TS-M009-1 HLA-A*24:02 EBV Mix Tetramer-PE

HIV

TS-M027-1 HLA-A*02:01 HIV gag Tetramer-SLYNTVATL-PE
TS-0008-1C HLA-A*02:01 HIV pol Tetramer-ILKEPVHGV-PE
TS-M007-1 HLA-A*24:02 HIV env Tetramer-RYLRDQQLL-PE
TS-M054-1 HLA-B*07:02 HIV nef Tetramer-TPGPGVRYPL-PE
TS-M106-1 HLA-B*35:01 HIV nef Tetramer-VPLRPMTY-PE
TS-M055-1 HLA-B*35:01 HIV RT Tetramer-NPDIVIYQY-PE

HBV

TS-0018-1C HLA-A*02:01 HBV core Tetramer-FLPSDFFPSV-PE
TS-0022-1C HLA-A*24:02 HBV core Tetramer-EYLVSFGVW-PE
TS-0023-1C HLA-A*24:02 HBV pol Tetramer-KYTSFPWLL-PE

Adenovirus

TS-M058-1 HLA-A*02:01 AdV Hexon$_{913-921}$ Tetramer-YLLFEVFDV-PE
TS-M059-1 HLA-A*02:01 AdV Hexon$_{914-922}$ Tetramer-LLFEVFDVV-PE
TS-M061-1 HLA-A*02:01 AdV Hexon$_{917-925}$ Tetramer-YVLFEVFDV-PE
TS-M062-1 HLA-A*24:02 AdV Hexon$_{37-45}$ Tetramer-TYFNLGNKF-PE
TS-M063-1 HLA-A*24:02 AdV Hexon$_{37-45}$ Tetramer-TYFSLNNKF-PE
TS-M064-1 HLA-A*24:02 AdV Hexon$_{896-704}$ Tetramer-VYSGSIPYL-PE
TS-M065-1 HLA-B*07:02 AdV Hexon$_{114-124}$ Tetramer-KPYSGTAYNSL-PE
TS-M066-1 HLA-B*07:02 AdV Hexon$_{114-124}$ Tetramer-KPYSGTAYNAL-PE
TS-M067-1 HLA-B*35:01 AdV Hexon$_{320-329}$ Tetramer-MPNRPNYIAF-PE
TS-M068-1 HLA-B*35:01 AdV Hexon$_{705-713}$ Tetramer- IPYLDGTFY-PE

HPV

TS-0031-1 HLA-A*02:01 HPV E7 Tetramer-YMLDLQPET-PE

VSV

TS-M122-1 HLA-A*02:01 VZV IE62 Tetramer-ALWALPHAA-PE

Influenza

TS-0012-1 HLA-A*02:01 Influenza M1 Tetramer-GILGFVFTL-PE

Mycobacterium tuberculosis

TS-M026-1 HLA-A*02:01 MPT51 Tetramer-TLAGKGISVV-PE
TS-M127-1 HLA-A*02:01 Mtb Rv1614 Tetramer-FLYELIWNV-PE

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

Others

TS-HCD-1 Human CD1d Tetramer-PE
TS-HCD-2 Human CD1d Tetramer-APC

T-Select Peptides

TS-0010-P HLA-A*02:01 CMV pp65 peptide
TS-0020-P HLA-A*24:02 CMV pp65 peptide
TS-0012-P HLA-A*02:01 Influenza M1 peptide
TS-M001-P HLA-A*24:02 EBV LMP2 peptide
TS-M002-P HLA-A*24:02 EBV BRLF1 peptide
TS-M003-P HLA-A*24:02 EBV BMLF1 peptide
TS-M004-P HLA-A*24:02 EBV EBNA3A peptide
TS-M005-P HLA-A*24:02 EBV EBNA3B peptide
TS-M007-P HLA-A*24:02 HIV env gp160 peptide
TS-M011-P HLA-A*02:01 NY-ESO-1 peptide
TS-M012-P HLA-A*11:01 CMV pp65 peptide
TS-M017-P HLA-A*02:01 HTLV-1 Tax$_{11-19}$ peptide
TS-M018-P HLA-A*24:02 HTLV-1 Tax$_{301-309}$ peptide
TS-M025-P HLA-A*24:02 survivin-2B peptide
TS-M026-P HLA-A*02:01 MPT51 peptide
TS-M027-P HLA-A*02:01 HIV gag 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)

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