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

T-Select MHC Tetramer

HLA-A*24:02 Influenza PB1$_{482-490}$ Tetramer-SYINRTGTF (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.

Influenza viruses are grouped into 3 major types (A, B, and C), and strains are further divided into multiple subtypes based on the virus surface proteins hemagglutinin (HA) and neuraminidase (NA). Influenza rapidly spreads around the world in seasonal epidemics and imposes a considerable economic burden in the form of health care and hospitalization costs.

Many of the influenza vaccines are aimed at the induction of antibodies but not T cell responses. Since these vaccines mainly induce antibodies directed against the highly variable surface proteins HA and NA, protection efficacy is subtype or strain specific. On the other hand, cytotoxic T lymphocytes (CTLs) directed against internal influenza antigens (e.g. NP, M1, PA and PB1, PB2) are cross-reactive to various virus subtypes due to high conservancy of the antigens. Therefore, vaccination strategies focused on T cell immune responses are intensively developed as an alternative to the one based on the induction of the type-specific *neutralizing antibodies*.

These Tetramer reagents comprise human class I HLA-A*24:02 and epitope peptide derived from the influenza A virus RNA polymerase basic protein 1 (PB1), and they can detect HLA-A*24:02-restricted influenza PB1-specific $CD8^+$ T cells.

A Tetramer, which is constructed with the same allele (HLA-A*24:02) of interest and an irrelevant peptide, may be used as a negative control Tetramer.

Storage Conditions

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

HLA Restriction: HLA-A*24:02

Origin and Sequence of CTL Epitope

Influenza A virus
A/Fort Monmouth/1/1947-mouse adapted(H1N1)
A/Hong Kong/516/97(H5N1)
A/swine/Shandong/fZC/2003(H9N2)

PB1 (482-490 aa, SYINRTGTF)

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-M147-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M147-2
Streptavidin-Allophycocyanin (SA-APC)
Excites at 633-635 nm
Emits at 660-680 nm

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).

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Usage

This reagent is for use with standard flow cytometry methodologies.

References for Products

1) Staneková Z & Varečková E, *et al. Virol J* **7**: 351 (2010)
2) Ichihashi T, *et al. PLoS One* **6**: e24626 (2011)
3) Uchida T, *Microbiol Immunol* **55**: 19-27 (2011)
4) Huber SKR, *et al. PLoS One* **10**: e0127969 (2015)

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
- 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. 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 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. 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 CD8 antibody, clone SFCI21Thy2D3 (T8, Beckman Coulter, Inc.), which does not block or interfere with the specific binding of MHC Tetramers to T cells.
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

Influenza Tetramers for Human

TS-M045-1	HLA-A*01:01	Influenza NP		Tetramer-CTELKLSDY-PE
TS-M046-1	HLA-B*35:01	Influenza NP		Tetramer-LPFEKSTVM-PE
TS-0012-1C	HLA-A*02:01	Influenza M1		Tetramer-GILGFVFTL-PE
TS-M144-1	HLA-A*24:02	influenza	$PA_{130-138}$	Tetramer-PE
TS-M145-1	HLA-A*24:02	influenza	$PB1_{216-224}$	Tetramer-PE
TS-M146-1	HLA-A*24:02	influenza	$PB1_{430-438}$	Tetramer-PE
TS-M147-1	HLA-A*24:02	influenza	$PB1_{482-490}$	Tetramer-PE
TS-M148-1	HLA-A*24:02	influenza	$PB1_{498-505}$	Tetramer-PE
TS-M149-1	HLA-A*24:02	influenza	$PB2_{549-557}$	Tetramer-PE

Class II Tetramers for Human

TS-M801-1	HLA-DRB1*01:01	human $CLIP_{103-117}$	Tetramer-PE
TS-M802-1	HLA-DRB1*01:01	HIV $gag_{295-307}$	Tetramer-PE
TS-M804-1	HLA-DRB1*01:01	Influenza $HA_{306-318}$	Tetramer-PE
TS-M805-1	HLA-DRB1*04:05	human $CLIP_{103-117}$	Tetramer-PE
TS-M806-1	HLA-DRB1*04:05	Influenza $HA_{306-318}$	Tetramer-PE
TS-M807-1	HLA-DRB1*11:01	human $CLIP_{103-117}$	Tetramer-PE
TS-M808-1	HLA-DRB1*11:01	Influenza $HA_{306-318}$	Tetramer-PE
TS-M809-1	HLA-DRB1*04:01	human $CLIP_{103-117}$	Tetramer-PE
TS-M810-1	HLA-DRB1*04:01	Influenza $HA_{306-318}$	Tetramer-PE

Influenza Tetramers for Mouse

TS-M502-1	$H\text{-}2D^b$	Influenza NP Tetramer-ASNENMDTM-PE
TS-M508-1	$H\text{-}2D^b$	Influenza NP Tetramer-ASNENMETM-PE
TS-M527-1	$H\text{-}2D^b$	Influenza NP Tetramer-ASNENMDAM-PE
TS-M528-1	$H\text{-}2D^b$	Influenza PA Tetramer-SSLENFRAYV-PE
TS-M533-1	$H\text{-}2K^b$	Influenza PB1 Tetramer-SSYRRPVGI-PE
TS-M520-1	$H\text{-}2K^d$	Influenza HA Tetramer-IYSTVASSL-PE
TS-M535-1	$H\text{-}2K^d$	Influenza HA Tetramer-LYQNVGTYV-PE
TS-M534-1	$H\text{-}2K^d$	Influenza NP Tetramer-TYQRTRALV-PE

Peptides

TS-M007-P	HLA-A*24:02 HIV env gp160 peptide
TS-0012-P	HLA-A*02:01 Influenza M1 peptide
TS-0029-P	HLA-A*02:01 Negative peptide
TS-M502-P	$H\text{-}2D^b$ Influenza NP peptide
TS-M508-P	$H\text{-}2D^b$ Influenza NP peptide
TS-M527-P	$H\text{-}2D^b$ Influenza NP peptide
TS-M528-P	$H\text{-}2D^b$ Influenza PA peptide
TS-M520-P	$H\text{-}2K^d$ Influenza HA peptide
TS-M534-P	$H\text{-}2K^d$ Influenza NP peptide
TS-M804-P	Influenza $HA_{306-318}$ peptide
TS-M801-P	Human $CLIP_{103-117}$ peptide
TS-M802-P	HLA-DRB1*01:01 HIV $gag_{295-307}$ peptide

Kits

4844	IMMUNOCYTO CD107a Detection Kit
AM-1005M	IMMUNOCYTO Cytotoxicity Detection Kit

Others

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

PBMCs from healthy HLA-A*24:02-positive donors were collected from freshly isolated heparinized peripheral blood according to standard methods.

Aliquots of the PBMCs (1 x 10^6 cells) were stained with MHC Tetramers, CD8 antibody, and 7-AAD as day 0.

Another aliquots of PBMCs (2 x 10^6 cells/mL) were incubated in culture tubes (Round-Bottom Tube, BD, PN 352059) in the presence of a synthetic peptide (10 μg/mL) and 5% (v/v) autologous plasma. After 48 h, an equal volume of medium containing 100 U/mL interleukin-2 (IL-2) was added to each culture tube, and every 2 to 3 days thereafter half of the medium was replaced with fresh medium containing IL-2 (50 U/mL). At the day 14 or 15, aliquots of these cells were stained with MHC Tetramers, CD8 antibody, and 7-AAD.

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 donors underlined are who have caught influenza within a year.

HLA-A*24:02-restricited influenza PB1-specific CTLs were not detectable in the freshly isolated PBMCs of all donors. On the other hand, after 14-15 days of *in vitro* peptide stimulation, the CTLs were detected in 1 out of 4 donors.