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

T-Select MHC Tetramer

HLA-A*02:01 Influenza M1 Tetramer -GILGFVFTL (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 epitope peptide derived from the influenza A virus matrix protein 1 (M1), and it can detect HLA-A*02:01-restricted influenza M1-specific $CD8^+$ T cells.

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 and neuraminidase. Influenza rapidly spreads around the world in seasonal epidemics and imposes a considerable economic burden in the form of health care and hospitalization costs. Mouse model is useful for investigating immune responses to influenza virus infection and the development of more effective influenza vaccines.

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.

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*02:01

Origin and Sequence of CTL Epitope

Influenza A/X31 (H3N2),
Influenza A/Puerto Rico/8/1934 (PR8, H1N1)
Matrix protein 1 (M1, 58-66 aa, GILGFVFTL)

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-0012-1C
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-0012-2C
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).

Usage

This reagent is for use with standard flow cytometry methodologies.

References for Products

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23) Harari A, *et al. J Virol* **83**: 2862-2871 (2009)
24) Alanio C, *et al. Blood* **115**: 3718-3725 (2010)
25) Cellerai C, *et al*. *J Virol* **84**: 3868–3878 (2010)
26) Tu W, *et al. J Virol* **84**: 6527-6535 (2010)
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28) Romano E, *et al*. *Clin Cancer Res* **17**: 1984-1997 (2011)
29) Odumade OA, *et al*. *J Exp Med* **209**: 471-478 (2012)
30) Flamar A-L, *et al*. *J Immunol* **189**: 2645-2655 (2012)

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 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

Influenza Tetramers for Human

TS-M045-1	HLA-A*01:01 Influenza NP Tetramer-CTELKLSDY-PE
TS-M045-2	HLA-A*01:01 Influenza NP Tetramer-CTELKLSDY-APC
TS-M046-1	HLA-B*35:01 Influenza NP Tetramer-LPFEKSTVM-PE
TS-M046-2	HLA-B*35:01 Influenza NP Tetramer-LPFEKSTVM-APC
TS-0012-1C	HLA-A*02:01 Influenza M1 Tetramer-GILGFVFTL-PE
TS-0012-2C	HLA-A*02:01 Influenza M1 Tetramer-GILGFVFTL-APC

Influenza Tetramers for Mouse

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

Peptides

TS-0012-P	HLA-A*02:01 Influenza M1 peptide
TS-0029-P	HLA-A*02:01 Negative peptide
TS-M502-P	H-2D^b Influenza NP peptide
TS-M508-P	H-2D^b Influenza NP peptide
TS-M527-P	H-2D^b Influenza NP peptide
TS-M520-P	H-2K^d Influenza HA peptide
TS-M528-P	H-2D^b Influenza PA peptide
TS-M701-P	I-A^b HBc helper peptide
TS-M702-P	I-A^d Tetanus toxin p30 helper peptide
TS-M703-P	I-A^b/I-A^d OVA helper peptide
TS-M704-P	I-A^b MOG_{35-55} peptide
TS-M708-P	I-A^k HEL peptide

Kit

AM-1005	IMMUNOCYTO Cytotoxity Detection Kit

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

PBMCs from healthy donors were collected from freshly isolated heparinized peripheral blood according to standard methods. HLA serotyping was performed by staining with FITC-conjugated anti-HLA-A2 (clone BB7.2, MBL, PN K0186-4) and its isotype control antibody (MBL, PN M077-4), then genotyping for HLA-A2 was performed.

Aliquots of the PBMCs ($1x10^6$ cells) of HLA-A2 positive donors were stained with MHC Tetramers and CD8 antibody. Numbers in the top right quadrants represent the percentage of MHC Tetramer-positive cells in the total $CD8^+$ cells.

HLA-A*02:01 Influenza M1 Tetramer-positive CTLs were detected in freshly isolated PBMCs. However, CMV IE1-specific CTLs were not detected by HLA-A*02:01 CMV IE1 Tetramer (MBL, PN TS-M057-1) staining. Staining results of donor C and D show that HLA-A*02:01 Influenza M1 Tetramer is a potential tool for monitoring Influenza M1-specific CTLs not only in individuals with HLA-A*02:01 but also in those with HLA-A*02:06 and HLA-A*02:07 genotypes.