

**T-Select MHC Tetramer**

**HLA-DRB1\*04:05 human CLIP<sub>103-117</sub> Tetramer  
-PVSKMRMATPLLMQA (20 tests)**

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

**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 CD4<sup>+</sup> antigen-specific T cells requires cognate recognition of the T cell receptor (TCR) by a class II MHC/peptide complex. This can be done using T-Select MHC class II Tetramers which are composed of four MHC class II 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 CD4<sup>+</sup> 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 II HLA-DRB1\*04:05 and epitope peptide CLIP derived from human class II associated Invariant chain (Ii).

CLIP is the part of the Ii, which is a chaperone molecule. In Antigen Presenting Cells (APCs), MHC class II molecules assemble in the ER with Ii. The CLIP segment of Ii binds the peptide binding groove of MHC class II molecules to protect the hydrophobic binding site. Following transport to the endosomal/lysosomal peptide-loading compartment, Ii is proteolytically cleaved, and the remaining CLIP peptide is exchanged with other peptides in a reaction catalyzed by HLA-DM.

This Tetramer can be used as a negative control Tetramer to different epitopes you are interested in of the same allele (HLA-DRB1\*04:05).

**HLA Restriction**

HLA-DRB1\*04:05

**Origin and Sequence of This Epitope**

Human class II associated Invariant chain peptide CLIP (103-117 aa, PVSKMRMATPLLMQA)

**References for This Product**

- 1) Paul A, *et al. Nature* **345**: 615-618 (1990)
- 2) Victor S, *et al. Nature* **375**: 802-806 (1995)
- 3) Sebastian A, *et al. J Exp Med* **181**: 1729-1741 (1995)
- 4) Lisa K, *et al. J Cell* **82**: 155-165 (1995)
- 5) Felix B, *et al. PNAS USA* **98**: 12168-12173 (2001)
- 6) Cheryl LD, *et al. J Clin Invest* **112**: 831-842 (2003)
- 7) Gerald TN, *et al. J Immunol* **188**: 2477-2482 (2012)

**Reagents**

200 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<sub>3</sub>.

**Conjugates**

TS-M805-1

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

TS-M805-2

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.

**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 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 acidic 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-CD4-FITC, Beckman Coulter, Inc., PN A07750
- 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  $\mu$ L of T-Select MHC Tetramer to each 12 x 75 mm test tube.
3. Add 200  $\mu$ L 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-CD4) 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  $2 \times 10^7$  cells/mL. 50  $\mu$ L of sample is required for each T-Select MHC Tetramer determination.
2. Add 10  $\mu$ L of Clear Back (human FcR blocking reagent, MBL, PN MTG-001) to each 12 x 75 mm test tube.
3. Add 50  $\mu$ L PBMC into each test tube (e.g.  $1 \times 10^6$  cells per tube).
4. Incubate for 5 minutes at room temperature.
5. Add 10  $\mu$ L 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-CD4) 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%  $\text{NaN}_3$ /PBS).
10. Centrifuge tubes at 400 x g for 5 minutes.
11. Aspirate or decant the supernatant.
12. Resuspend the pellet in 500  $\mu$ L 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. 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.
- E. 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).
- F. 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 class II Tetramers

TS-M801-1 HLA-DRB1\*01:01 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M801-2 HLA-DRB1\*01:01 human CLIP<sub>103-117</sub> Tetramer-APC  
TS-M802-1 HLA-DRB1\*01:01 HIV gag<sub>295-307</sub> Tetramer-PE  
TS-M802-2 HLA-DRB1\*01:01 HIV gag<sub>295-307</sub> Tetramer-APC  
TS-M803-1 HLA-DRB1\*01:01 EBV EBNA1<sub>515-527</sub> Tetramer-PE  
TS-M803-2 HLA-DRB1\*01:01 EBV EBNA1<sub>515-527</sub> Tetramer-APC  
TS-M804-1 HLA-DRB1\*01:01 Influenza HA<sub>306-318</sub> Tetramer-PE  
TS-M804-2 HLA-DRB1\*01:01 Influenza HA<sub>306-318</sub> Tetramer-APC  
TS-M805-1 HLA-DRB1\*04:05 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M805-2 HLA-DRB1\*04:05 human CLIP<sub>103-117</sub> Tetramer-APC  
TS-M806-1 HLA-DRB1\*04:05 Influenza HA<sub>306-318</sub> Tetramer-PE  
TS-M806-2 HLA-DRB1\*04:05 Influenza HA<sub>306-318</sub> Tetramer-APC  
TS-M807-1 HLA-DRB1\*11:01 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M807-2 HLA-DRB1\*11:01 human CLIP<sub>103-117</sub> Tetramer-APC  
TS-M808-1 HLA-DRB1\*11:01 Influenza HA<sub>306-318</sub> Tetramer-PE  
TS-M808-2 HLA-DRB1\*11:01 Influenza HA<sub>306-318</sub> Tetramer-APC  
TS-M809-1 HLA-DRB1\*04:01 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M809-2 HLA-DRB1\*04:01 human CLIP<sub>103-117</sub> Tetramer-APC  
TS-M810-1 HLA-DRB1\*04:01 Influenza HA<sub>306-318</sub> Tetramer-PE  
TS-M810-2 HLA-DRB1\*04:01 Influenza HA<sub>306-318</sub> Tetramer-APC

#### T-Select Mouse class II Tetramers

TS-M704-1 I-A<sup>D</sup> MOG<sub>35-55</sub> Tetramer-PE  
TS-M704-2 I-A<sup>D</sup> MOG<sub>35-55</sub> Tetramer-APC  
TS-M705-1 I-A<sup>D</sup> FMLV<sub>123-141</sub> Tetramer-PE  
TS-M706-1 I-A<sup>D</sup> E $\alpha$ <sub>52-68</sub> Tetramer-PE  
TS-M707-1 I-A<sup>D</sup> ESAT-6<sub>1-20</sub> Tetramer-PE  
TS-M710-1 I-A<sup>D</sup> OVA<sub>323-339</sub> Tetramer-PE  
TS-M710-2 I-A<sup>D</sup> OVA<sub>323-339</sub> Tetramer-APC

#### T-Select Peptides

TS-M701-P I-A<sup>D</sup> HbC helper peptide  
TS-M702-P I-A<sup>D</sup> Tetanus toxin p30 helper peptide  
TS-M703-P I-A<sup>D</sup> OVA<sub>323-339</sub> helper peptide  
TS-M704-P I-A<sup>D</sup> MOG<sub>35-55</sub> peptide  
TS-M707-P I-A<sup>D</sup> ESAT-6<sub>1-20</sub> peptide  
TS-M708-P I-A<sup>K</sup> HEL peptide  
TS-M801-P HLA-DRB1\*01:01 human CLIP<sub>103-117</sub> peptide  
TS-M802-P HLA-DRB1\*01:01 HIV gag<sub>295-307</sub> peptide  
TS-M803-P HLA-DRB1\*01:01 EBV EBNA1<sub>515-527</sub> peptide  
TS-M804-P HLA-DRB1\*01:01 Influenza HA<sub>306-318</sub> peptide

#### Kit

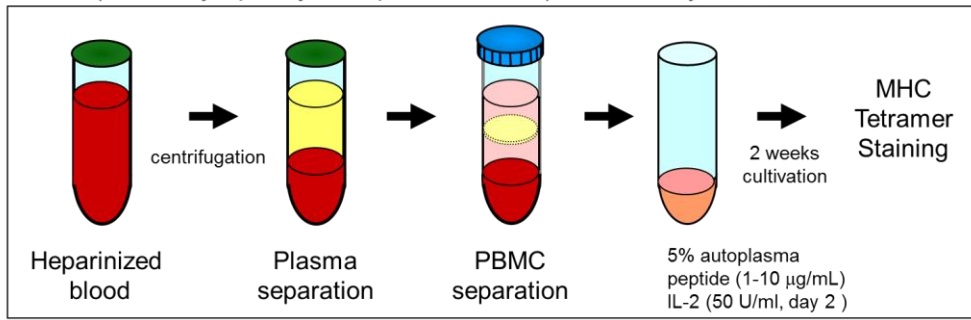
AM-1005M IMMUNOCYTO Cytotoxicity Detection Kit  
4844 IMMUNOCYTO CD107a Detection Kit  
8223 IMMUNOCYTO IFN- $\gamma$  ELISPOT Kit

#### Others

A07750 Anti-CD4 (Human) mAb-FITC  
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.

MLPC (Mixed-Lymphocyte-Peptide Cultures) modified by **MBL**

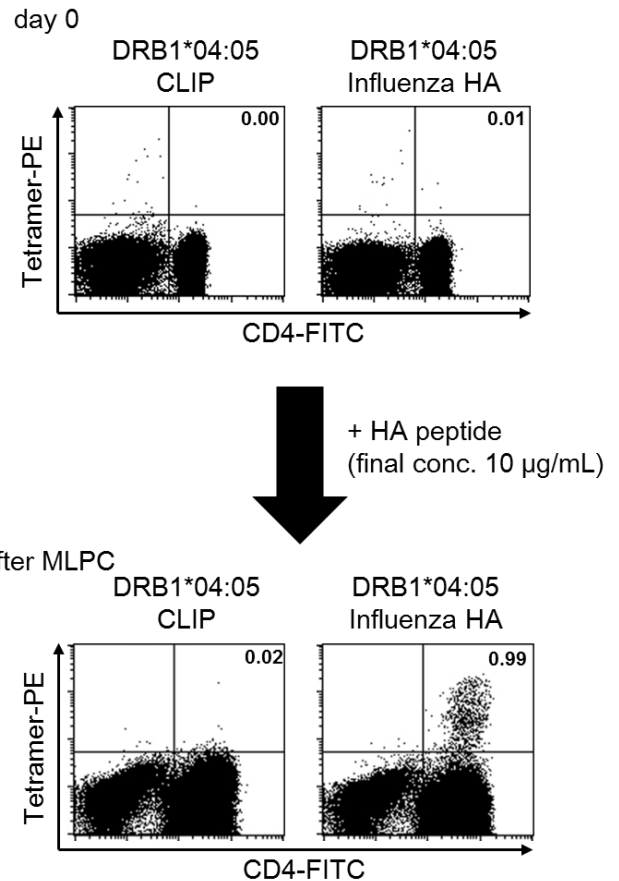


**Experimental Data**

PBMCs from HLA-DRB1\*04:05-positive healthy donors were collected from freshly isolated heparinized peripheral blood according to standard methods. Plasma was collected before PBMC separation by centrifugation at 3,000 rpm for 10 minutes, and stored at -30°C.

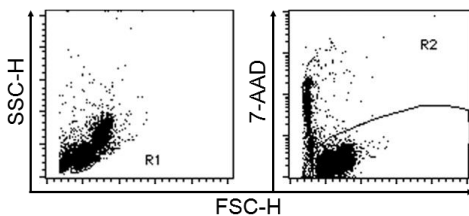
Aliquots of the PBMCs ( $1 \times 10^6$  cells) were stained with Influenza HA Tetramer (MBL, PN TS-M806-1) and CD4 antibody (day 0). Another aliquots of PBMCs ( $1-3 \times 10^6$  cells/mL) were incubated in culture tubes (Round-Bottom Tube, BD, PN 352059) in the presence of a synthetic peptide (10 µg/mL of PKYVKQNTLKLAT, TS-M804-P) 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). After 13 days, aliquots of these cells were stained with this MHC Tetramers, CD4 antibody, and 7-AAD (after MLPC).

The lymphocyte population was defined by an FSC/SSC gate (R1), and the viable cell population was defined by an FSC/7-AAD (R2). Data were analyzed by double gating on the lymphocyte and viable cell population (R1 and R2). Numbers in the top right quadrants represent the percentage of MHC Tetramer-positive cells in the total CD4<sup>+</sup> cells.

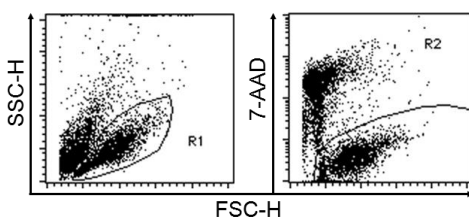


**Result**

day 0



after MLPC



To confirm specificity of MHC Tetramer staining, cells were stained with specific and negative control MHC Tetramer. DRB1\*04:05 human CLIP<sub>103-117</sub> Tetramer (MBL, PN TS-M805-1) was used as a negative control, containing the peptide PVSKMRMATPLLQA, CLIP.

Results showed that HLA-DRB1\*04:05-restricted CLIP Tetramer could be used as a negative Tetramer.

T-Select MHC Tetramers use patented technology (US patent No. 5,635,363, French application No. FR9911133, and Japanese patent No. P3506384) of Beckman Coulter, Inc..

MBL manufactures and distributes these products under license from Beckman Coulter, Inc..