

**T-Select MHC Tetramer**

# HLA-A\*24:02 CMV pp65 Tetramer -QYDPVAALF (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<sup>+</sup> 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<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 I HLA-A\*24:02 and epitope peptide derived from CMV pp65 structural protein, and it can detect HLA-A\*24:02-restricted CMV pp65-specific CD8<sup>+</sup> T cells by flow cytometry.

Cytomegalovirus (CMV/HHV-5) is a commonly widespread herpes virus that asymptotically infects most individuals. CMV-specific T cells in healthy individuals play an important role in the control of CMV reactivation. However, CMV infection in immunocompromised individuals is a major cause of infectious morbidity and mortality, especially among transplant recipients and those infected with the human immunodeficiency virus (HIV). The correlation between regeneration of CMV-specific T cells and protection from CMV disease has been shown in patients undergoing allogeneic bone marrow transplantation (BMT). Adoptive transfer of donor-derived CMV-specific T cells has led to restoration of virus-specific immunity in BMT patients

with reducing the rate of viral reactivation. Monitoring of CMV-specific T cells using MHC Tetramer is useful for predicting patients at risk for recurrent or persistent CMV infection, CMV disease, or transplant related mortality.

## HLA Restriction

HLA-A\*24:02

## Origin and Sequence of CTL Epitope

CMV pp65 (341-349 aa, QYDPVAALF)

## 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<sub>3</sub>.

## Conjugates

TS-0020-1C

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

TS-0020-2C

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 This Product

- 1) Kuzushima K, *et al. Blood* **98**: 1872-1881 (2001)
- 2) Kondo E, *et al. J Immunol* **169**: 2164-2171 (2002)
- 3) Kuzushima K, *et al. Blood* **101**: 1460-1468 (2003)
- 4) Kondo E, *et al. Blood* **103**: 630-638 (2004)
- 5) Watanabe K, *et al. Int J Hematol* **88**: 311-320 (2008)
- 6) Kozako T, *et al. Leukemia* **23**: 375-382 (2009)

## 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  $\alpha 3$  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  $\mu$ 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-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  $2 \times 10^7$  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-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<sub>3</sub>/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. We recommend the use of anti-CD8 antibody, clone SFC121Thy2D3 (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

#### CMV

TS-M012-1	HLA-A*11:01	CMV pp65 Tetramer-ATVQQGNLQK-PE
TS-0010-1C	HLA-A*02:01	CMV pp65 Tetramer-NLVPMVATV-PE
TS-0010-2C	HLA-A*02:01	CMV pp65 Tetramer-NLVPMVATV-APC
TS-0020-1C	HLA-A*24:02	CMV pp65 Tetramer-QYDPVAALF-PE
TS-0020-2C	HLA-A*24:02	CMV pp65 Tetramer-QYDPVAALF-APC
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-0027-2C	HLA-B*35:01	CMV pp65 Tetramer-IPSINVHHY-APC
TS-0025-1C	HLA-B*07:02	CMV pp65 Tetramer-TPRVTGGGAM-PE
TS-0025-2C	HLA-B*07:02	CMV pp65 Tetramer-TPRVTGGGAM-APC
TS-M099-1	HLA-B*07:02	CMV pp65 Tetramer-RPHERNGFTVL-PE
TS-M099-2	HLA-B*07:02	CMV pp65 Tetramer-RPHERNGFTVL-APC
TS-0024-1C	HLA-A*01:01	CMV pp50 Tetramer-VTEHDTLLY-PE
TS-0024-2C	HLA-A*01:01	CMV pp50 Tetramer-VTEHDTLLY-APC
TS-M057-1	HLA-A*02:01	CMV IE1 Tetramer-VLEETSVML-PE
TS-M057-2	HLA-A*02:01	CMV IE1 Tetramer-VLEETSVML-APC
TS-M100-1	HLA-A*03:01	CMV IE1 Tetramer-KLGGALQAK-PE
TS-M100-2	HLA-A*03:01	CMV IE1 Tetramer-KLGGALQAK-APC
TS-0026-1C	HLA-B*08:01	CMV IE1 Tetramer-ELRRKMMYM-PE
TS-0026-2C	HLA-B*08:01	CMV IE1 Tetramer-ELRRKMMYM-APC

#### HIV

TS-M027-1	HLA-A*02:01	HIV gag Tetramer-SLYNTVATL-PE
TS-M027-2	HLA-A*02:01	HIV gag Tetramer-SLYNTVATL-APC
TS-M027-3	HLA-A*02:01	HIV gag Tetramer-SLYNTVATL-FITC
TS-0008-1C	HLA-A*02:01	HIV pol Tetramer-ILKEPVHGV-PE
TS-M007-1	HLA-A*24:02	HIV env Tetramer-RYLDRDQQL-PE
TS-M007-2	HLA-A*24:02	HIV env Tetramer-RYLDRDQQL-APC
TS-M007-3	HLA-A*24:02	HIV env Tetramer-RYLDRDQQL-FITC
TS-M054-1	HLA-B*07:02	HIV nef Tetramer-TPGPGVRYPL-PE
TS-M054-2	HLA-B*07:02	HIV nef Tetramer-TPGPGVRYPL-APC
TS-M106-1	HLA-B*35:01	HIV nef Tetramer-VPLRPMTY-PE
TS-M055-1	HLA-B*35:01	HIV RT Tetramer-NPDIVYQY-PE

#### T-Select Peptides

TS-0010-P	HLA-A*02:01	CMV pp65 peptide
TS-0020-P	HLA-A*24:02	CMV pp65 peptide
TS-M007-P	HLA-A*24:02	HIV env gp160 peptide
TS-M012-P	HLA-A*11:01	CMV pp65 peptide
TS-M027-P	HLA-A*02:01	HIV gag peptide

#### Others

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

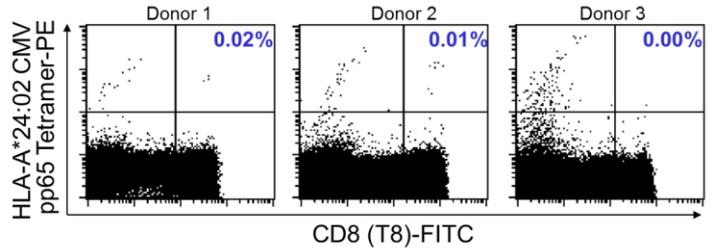
**Experimental Data**

PBMCs from HLA-A\*24:02-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.

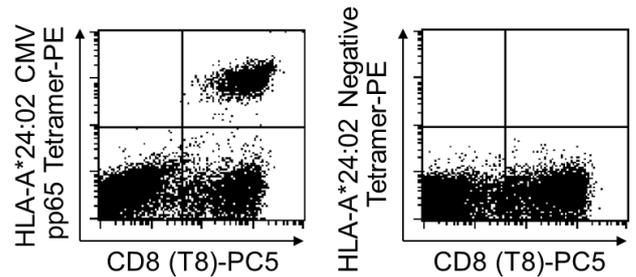
Aliquots of the PBMCs ( $1 \times 10^6$  cells) were stained with MHC Tetramers and CD8 antibody (Results 1). Another aliquots of PBMCs ( $1-3 \times 10^6$  cells/mL) were incubated in culture tubes (Round-Bottom Tube, BD, PN 352059) in the presence of a synthetic peptide (10 µg/mL of QYDPVAALF, MBL, PN TS-0020-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). At the indicated time points, aliquots of these cells were stained with MHC Tetramers, CD8 antibody, and 7-AAD (Results 2, 3). Numbers in the top right quadrants represent the percentage of MHC Tetramer-positive cells in the total CD8<sup>+</sup> cells.

Flow cytometric enumeration of CMV pp65-specific CD8<sup>+</sup> T lymphocytes in healthy individuals reveals that HLA-A\*24:02-restricted CMV pp65-specific CTLs were very rare compared the frequency of HLA-A2-restricted CMV pp65-specific CTLs. However, HLA-A\*24:02-restricted CMV pp65-specific CTLs were detectable in the peptide stimulated PBMCs of all healthy donors we tested (n=18).

Results 1: Tetramer staining of freshly isolated PBMCs

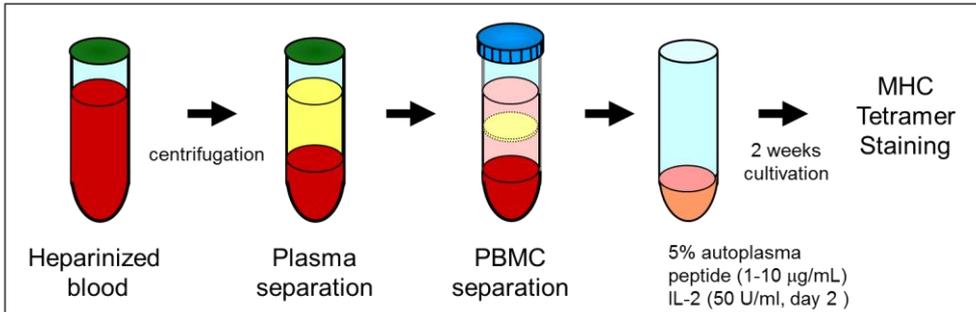


Results 2: Tetramer staining of peptide-stimulated PBMCs for 14 days



To confirm specificity of MHC Tetramer staining, cells were stained with specific and negative control MHC Tetramer (MBL, PN TS-M007-1) containing the peptide RYL RDQQLL, derived from the human immunodeficiency virus envelope (HIV env) protein.

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



Results 3: Expansion of CMV pp65-specific CTLs by MLPC

