

T-Select MHC Tetramer

# HLA-A\*02:01 HHV-6B U54 Tetramer -ILYGPLTRI (50 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 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\*02:01 and epitope peptide derived from HHV-6B U54 protein, and it can detect HLA-A\*02:01-restricted HHV-6B U54-specific CD8<sup>+</sup> T cells by flow cytometry.

Human herpesvirus 6 (HHV-6) is a  $\beta$ -herpesvirus and exists as 2 species, HHV-6A and HHV-6B. HHV-6B, the more widespread species, is a pathogen infecting more than 90% of individuals world-wide during childhood and then establishing a latent and lifelong infection. HHV-6B reactivation occurs in its immunocompromised host (e.g. patients receiving allogeneic hematopoietic stem cell or solid-organ transplantation and AIDS patients) and causes several severe complications. Recently, there have been notable advances in defining T cell responses specific to HHV-6 and in developing approaches to adoptive immunotherapy. Monitoring of HHV-6B-specific T cells using MHC Tetramer is useful for understanding of these T cell responses.

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.

## HLA Restriction

HLA-A\*02:01

## Origin and Sequence of CTL Epitope

Human herpesvirus-6 variant B (HHV-6B) (Strain Z29) U54 protein (129-137 aa, ILYGPLTRI)

## Reference for HHV-6B and This Epitope

- 1) Martin LK, *et al. Eur J Immunol* **42**:2901-2912 (2012)
- 2) Becerra A, *et al. Curr Opin Virol* **9**:154-161 (2014)

## Conjugates

TS-M143-1

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

TS-M143-2

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

## Reagents

500  $\mu$ L liquid - 10  $\mu$ L/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>.

## 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.

## 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

##### Adenovirus

TS-M058-1	HLA-A*02:01 Adv11 Hexon <sub>913-921</sub> Tetramer-YLLFEVFDV-PE
TS-M059-1	HLA-A*02:01 Adv11 Hexon <sub>914-922</sub> Tetramer-LLFEVFDVV-PE
TS-M062-1	HLA-A*24:02 Adv11 Hexon <sub>37-45</sub> Tetramer-TYFNLGNKF-PE
TS-M064-1	HLA-A*24:02 Adv11 Hexon <sub>696-704</sub> Tetramer-VYSGSIPYL-PE
TS-M063-1	HLA-A*24:02 Adv5 Hexon <sub>137-45</sub> Tetramer-TYFSLNKNKF-PE
TS-M061-1	HLA-A*02:01 Adv Hexon <sub>917-925</sub> Tetramer-YVLFVFDV-PE
TS-M067-1	HLA-B*35:01 Adv Hexon <sub>320-329</sub> Tetramer-MPNRPNYIAF-PE
TS-M068-1	HLA-B*35:01 Adv Hexon <sub>705-713</sub> Tetramer-IPYLDGTFY-PE
TS-M065-1	HLA-B*07:02 Adv Hexon <sub>114-124</sub> Tetramer-KPYSGTAYNSL-PE
TS-M066-1	HLA-B*07:02 Adv Hexon <sub>114-124</sub> Tetramer-KPYSGTAYNAL-PE

##### CMV

TS-M057-1	HLA-A*02:01 CMV IE1 <sub>316-324</sub> Tetramer-VLEETSVML-PE
TS-M100-1	HLA-A*03:01 CMV IE1 <sub>184-192</sub> Tetramer-KLGGALQAK-PE
TS-0026-1C	HLA-B*08:01 CMV IE1 Tetramer-ELRRKMMYM-PE

TS-0024-1C	HLA-A*01:01 CMV pp50 Tetramer-VTEHDTLLY-PE
TS-0010-1C	HLA-A*02:01 CMV pp65 Tetramer-NLVP/MVATV-PE
TS-0020-1C	HLA-A*24:02 CMV pp65 Tetramer-QYDPVAAALF-PE
TS-M012-1	HLA-A*11:01 CMV pp65 Tetramer-ATVQGGQNLK-PE
TS-M099-1	HLA-B*07:02 CMV pp65 Tetramer-RPHERNGFTVL-PE
TS-0025-1C	HLA-B*07:02 CMV pp65 Tetramer-TPRV/TGGGAM-PE
TS-0027-1C	HLA-B*35:01 CMV pp65 Tetramer-IPSINVHHY-PE
TS-M013-1	HLA-B*15:01 CMV pp65 Tetramer-KMQVIGDQY-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-M002-1	HLA-A*24:02 EBV BRLF1 Tetramer-TYPVLEEMF-PE
TS-M124-1	HLA-A*03:01 EBV BRLF1 Tetramer-RVRAYTYSK-PE
TS-M036-1	HLA-B*08:01 EBV BZLF1 <sub>190-197</sub> Tetramer-RAKFKQLL-PE
TS-M037-1	HLA-B*35:01 EBV BZLF1 <sub>154-64</sub> Tetramer-EPLPQGLTAY-PE
TS-M033-1	HLA-A*03:01 EBV EBNA3A <sub>636-611</sub> Tetramer-RLRAEAQVK-PE
TS-M004-1	HLA-A*24:02 EBV EBNA3A Tetramer-RYSIFFDYM-PE
TS-M142-1	HLA-B*07:02 EBV EBNA3A Tetramer-RPPIFIRRL-PE
TS-M123-1	HLA-B*08:01 EBV EBNA3A Tetramer-FLRGRAYGL-PE
TS-M028-1	HLA-A*11:01 EBV EBNA3B <sub>399-408</sub> Tetramer-AVFDRKSDAK-PE
TS-M029-1	HLA-A*11:01 EBV EBNA3B <sub>416-424</sub> Tetramer-IVTDFSVIK-PE
TS-M005-1	HLA-A*24:02 EBV EBNA3B Tetramer-TYSAGIVQI-PE
TS-M006-1	HLA-A*02:01 EBV LMP1 Tetramer-YLQQNWWTL-PE
TS-M030-1	HLA-A*02:01 EBV LMP2 <sub>243-251</sub> Tetramer-TVCGGIMFL-PE
TS-M031-1	HLA-A*02:01 EBV LMP2 <sub>329-337</sub> Tetramer-LLWTLVLL-PE
TS-M069-1	HLA-A*02:01 EBV LMP2 <sub>356-364</sub> Tetramer-FLYALALLL-PE
TS-M032-1	HLA-A*02:01 EBV LMP2 <sub>426-434</sub> Tetramer-CLGGLLTMV-PE
TS-M034-1	HLA-A*24:02 EBV LMP2 <sub>131-139</sub> Tetramer-PYLFWLAAI-PE
TS-M001-1	HLA-A*24:02 EBV LMP2 Tetramer-IYVLVMLVL-PE
TS-M035-1	HLA-A*24:02 EBV LMP2 <sub>2419-427</sub> Tetramer-TYGPVFMSL-PE
TS-M038-1	HLA-B*35:01 EBV LMP2 <sub>1-9</sub> Tetramer-MGSLEMVPM-PE
TS-M111-1	HLA-A*11:01 EBV LMP2 Tetramer-SSCSCPLSK-PE
TS-M135-1	HLA-A*11:01 EBV LMP2 S9T Tetramer-SSCSCPLTK-PE
TS-M009-1	HLA-A*24:02 EBV Mix Tetramer

##### HIV

TS-M007-1	HLA-A*24:02 HIV env Tetramer-RYLDRDQQLL-PE
TS-M027-1	HLA-A*02:01 HIV gag <sub>77-85</sub> Tetramer-SLYNTVATL-PE
TS-M139-1	HLA-A*02:01 HIV gag <sub>19-27</sub> Tetramer-TLNAWVKVV-PE
TS-M110-1	HLA-A*24:02 HIV nef <sub>134-141</sub> Tetramer-RYPLTFGW-PE
TS-M054-1	HLA-B*07:02 HIV nef Tetramer-TPGPGVRYPL-PE
TS-M106-1	HLA-B*35:01 HIV nef <sub>74-81</sub> Tetramer-VPLRPMTY-PE
TS-0008-1C	HLA-A*02:01 HIV pol Tetramer-ILKEPVHGV-PE
TS-M055-1	HLA-B*35:01 HIV RT Tetramer-NPDIVYQY-PE

##### Control

TS-M007-1	HLA-A*24:02 Negative Tetramer-RYLDRDQQLL-PE
TS-M007-2	HLA-A*24:02 Negative Tetramer-RYLDRDQQLL-APC
TS-M007-3	HLA-A*24:02 Negative Tetramer-RYLDRDQQLL-FITC
TS-0029-1C	HLA-A*02:01 Negative Tetramer-PE
TS-0029-2C	HLA-A*02:01 Negative Tetramer-APC

##### Others

6603861	CD8-FITC (T8)
6607011	CD8-PC5 (T8)
A07704	7-AAD Viability Dye
MTG-001	Clear Back (Human FcR blocking reagent)
4844	IMMUNOCYTO CD107a Detection Kit
8223	IMMUNOCYTO IFN- $\gamma$ ELISPOT Kit
AM-1005M	IMMUNOCYTO Cytotoxicity Detection Kit

Please check our web site (<http://ruo.mbl.co.jp>) for up-to-date information on products and custom MHC Tetramers.

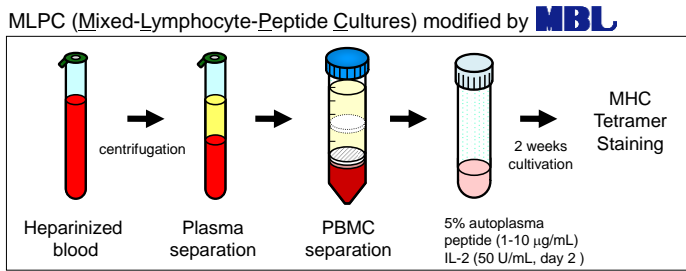
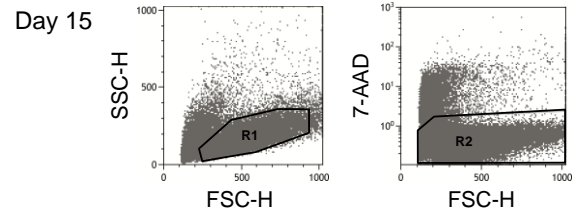


Fig. 1



### Experimental Data

PBMCs from HLA-A\*02:01-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  $-30^{\circ}\text{C}$ .

Aliquots of the PBMCs ( $1 \times 10^6$  cells) were stained with the following MHC Tetramers, FITC-labeled CD8 antibody, and 7-AAD (day 0).

- HLA-A\*02:01 HHV-6B U54 Tetramer (MBL, PN TS-M143-1)
- HLA-A\*02:01 HIV pol Tetramer (negative control) (MBL, PN TS-0008-1C)

Another aliquots of the PBMCs ( $1-3 \times 10^6$  cells in 1 mL of culture medium) were incubated in culture tubes (Round-Bottom Tube, BD, PN 352059) in the presence of a synthetic peptide ( $10 \mu\text{g/mL}$  of ILYGPLTRI; HHV-6B U54 protein, 129–137 aa) and 5% (v/v) autologous plasma. After 48 hours, 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 50 U/mL IL-2. On day 15, the cultured cells were collected and stained with the MHC Tetramers, FITC-labeled CD8 antibody, and 7-AAD.

### Result

The lymphocyte population was defined by an FSC/SSC gate (R1), and the viable cell population was defined by an FSC/7-AAD (R2) (Fig.1). 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<sup>+</sup> cells.

The HHV-6B U54-specific CTLs were induced from PBMCs of 2 donors (Donor A, B) among 4 donors (Fig.2).

Fig. 2

