

T-Select MHC class I human Tetramer

# HLA-A\*24:02 Adenovirus 11 Hexon<sub>696-704</sub>

## Tetramer-VYSGSIPYL (50 tests)

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

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

This T-Select MHC Tetramer uses patented technology (Japanese patent No. P4976294) of MBL.

### 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 in 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 the adenovirus type 11 (AdV11) Hexon protein, and it can detect HLA-A\*24:02-restricted AdV11 Hexon<sub>696-704</sub>-specific CD8<sup>+</sup> T cells by flow cytometry.

Adenovirus (AdV) infections prevalent in immunocompromised populations are crucial causes of morbidity and mortality. The AdV family consists of 51 known serotypes, distributed among six subgroups, from A to F. Subgroup A, B, and C serotypes are most frequently isolated from immunocompromised hosts and are the major causes of disease. In particular, infection with AdV type 11 of subgroup B frequently causes hemorrhagic cystitis (HC), which is a major complication in patients undergoing bone marrow transplantation and is the presenting sign of a lethal adenoviral infection. Unfortunately, there are as yet no approved antiviral

agents with proven efficacy for the treatment of severe AdV disease. An alternative promising therapeutic approach is immunotherapy by means of AdV-specific lymphocyte infusion, since several case reports have suggested that donor lymphocytes may contribute to the clearance of an AdV infection<sup>1), 2), 3)</sup>. CTL epitopes for AdV are valuable not only for monitoring antiviral immunity but also for application in adoptive immunotherapy, i.e. for *ex vivo* generation of antiviral CTLs, as has been successfully achieved for CMV or EBV<sup>4), 5)</sup>.

**HLA Restriction:** HLA-A\*24:02

### Origin and Sequence of CTL Epitope:

AdV11 Hexon (696-704 aa, VYSGSIPYL)

### References for This Product

- 1) Hromas R, *et al. Blood* **84**: 1689-1690 (1994)
- 2) Chakrabarti S, *et al. Bone Marrow Transplant* **26**: 305-307 (2000)
- 3) Leen AM, *et al. Blood* **103**: 1011-1019 (2004)
- 4) Walter EA, *et al. N Engl J Med* **333**: 1038-1044 (1995)
- 5) Heslop HE, *et al. Immunol Rev* **157**: 217-222 (1997)
- 6) Imahashi N, *et al. Mol Immunol* **56**: 399-405 (2013)

### 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)

## Reagents

T-Select MHC Class I Human Tetramer - 50 tests  
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-M064-1  
Streptavidin-Phycoerythrin (SA-PE)  
Excites at 486-580 nm  
Emits at 586-590 nm
- TS-M064-2  
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.

## 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
- MHC Tetramer Lyse Reagent, MBLI, PN T08002
- MHC Tetramer Fixative Reagent, MBLI, PN T08003
- Anti-CD8-FITC, Beckman Coulter, Inc., PN 6603861
- 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 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 2 mL of Lyse Reagent supplemented with 50 µL Fixative Reagent per tube.
9. Vortex for 5 seconds immediately after the addition of the Lyse/Fixative solution.
10. Incubate for a minimum of 10 minutes at room temperature protected from light.
11. Centrifuge tubes at 150 x g for 5 minutes.
12. Aspirate or decant the supernatant.
13. Add 3 mL of PBS and centrifuge tubes at 150 x g for 5 minutes.
14. Aspirate or decant the supernatant.
15. Resuspend the pellet in 500 µL of PBS with 0.1% formaldehyde. (12.5 µL Fixative Reagent/1 mL PBS).
16. 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 µL 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 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%  $\text{NaN}_3$ /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. (62.5  $\mu$ L Fixative Reagent/1 mL PBS).
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 AdV Hexon <sub>913-921</sub>	Tetramer-YLLFEVFDV-PE
TS-M058-2	HLA-A*02:01 AdV Hexon <sub>913-921</sub>	Tetramer-YLLFEVFDV-APC
TS-M059-1	HLA-A*02:01 AdV Hexon <sub>914-922</sub>	Tetramer-LLFEVFDV-PE
TS-M059-2	HLA-A*02:01 AdV Hexon <sub>914-922</sub>	Tetramer-LLFEVFDV-APC
TS-M061-1	HLA-A*02:01 AdV Hexon <sub>917-925</sub>	Tetramer-YVLFVFDV-PE
TS-M061-2	HLA-A*02:01 AdV Hexon <sub>917-925</sub>	Tetramer-YVLFVFDV-APC
TS-M062-1	HLA-A*24:02 AdV Hexon <sub>37-45</sub>	Tetramer-TYFNLGNKF-PE
TS-M062-2	HLA-A*24:02 AdV Hexon <sub>37-45</sub>	Tetramer-TYFNLGNKF-APC
TS-M063-1	HLA-A*24:02 AdV Hexon <sub>37-45</sub>	Tetramer-TYFSLNKKF-PE
TS-M063-2	HLA-A*24:02 AdV Hexon <sub>37-45</sub>	Tetramer-TYFSLNKKF-APC
TS-M064-1	HLA-A*24:02 AdV Hexon <sub>686-704</sub>	Tetramer-VYSGSIPYL-PE
TS-M064-2	HLA-A*24:02 AdV Hexon <sub>686-704</sub>	Tetramer-VYSGSIPYL-APC
TS-M065-1	HLA-B*07:02 AdV Hexon <sub>114-124</sub>	Tetramer-KPYSGTAYNSL-PE
TS-M065-2	HLA-B*07:02 AdV Hexon <sub>114-124</sub>	Tetramer-KPYSGTAYNSL-APC
TS-M066-1	HLA-B*07:02 AdV Hexon <sub>114-124</sub>	Tetramer-KPYSGTAYNAL-PE
TS-M066-2	HLA-B*07:02 AdV Hexon <sub>114-124</sub>	Tetramer-KPYSGTAYNAL-APC
TS-M067-1	HLA-B*35:01 AdV Hexon <sub>320-329</sub>	Tetramer-MPNRPNYAF-PE
TS-M067-2	HLA-B*35:01 AdV Hexon <sub>320-329</sub>	Tetramer-MPNRPNYAF-APC
TS-M068-1	HLA-B*35:01 AdV Hexon <sub>705-713</sub>	Tetramer-IPYLDGTFY-PE
TS-M068-2	HLA-B*35:01 AdV Hexon <sub>705-713</sub>	Tetramer-IPYLDGTFY-APC

#### Control

TS-0029-1C	HLA-A*02:01 Negative Tetramer-PE
TS-0029-2C	HLA-A*02:01 Negative Tetramer-APC
TS-M007-1	HLA-A*24:02 HIV env Tetramer-RYLRDQQLL-PE
TS-M007-2	HLA-A*24:02 HIV env Tetramer-RYLRDQQLL-APC
TS-M007-3	HLA-A*24:02 HIV env Tetramer-RYLRDQQLL-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

#### Others

4844	IMMUNOCYTO CD107a Detection Kit
8223	IMMUNOCYTO IFN- $\gamma$ ELISPOT Kit
AM-1005	IMMUNOCYTO Cytotoxicity Detection Kit
TS-8005	T-Select MHC IFN- $\gamma$ Kit
TS-9004	T-Select Antibody Gating Kit
TS-9017	T-Select MHC Tetramer T Cell Typing Kit

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

## Experimental Data

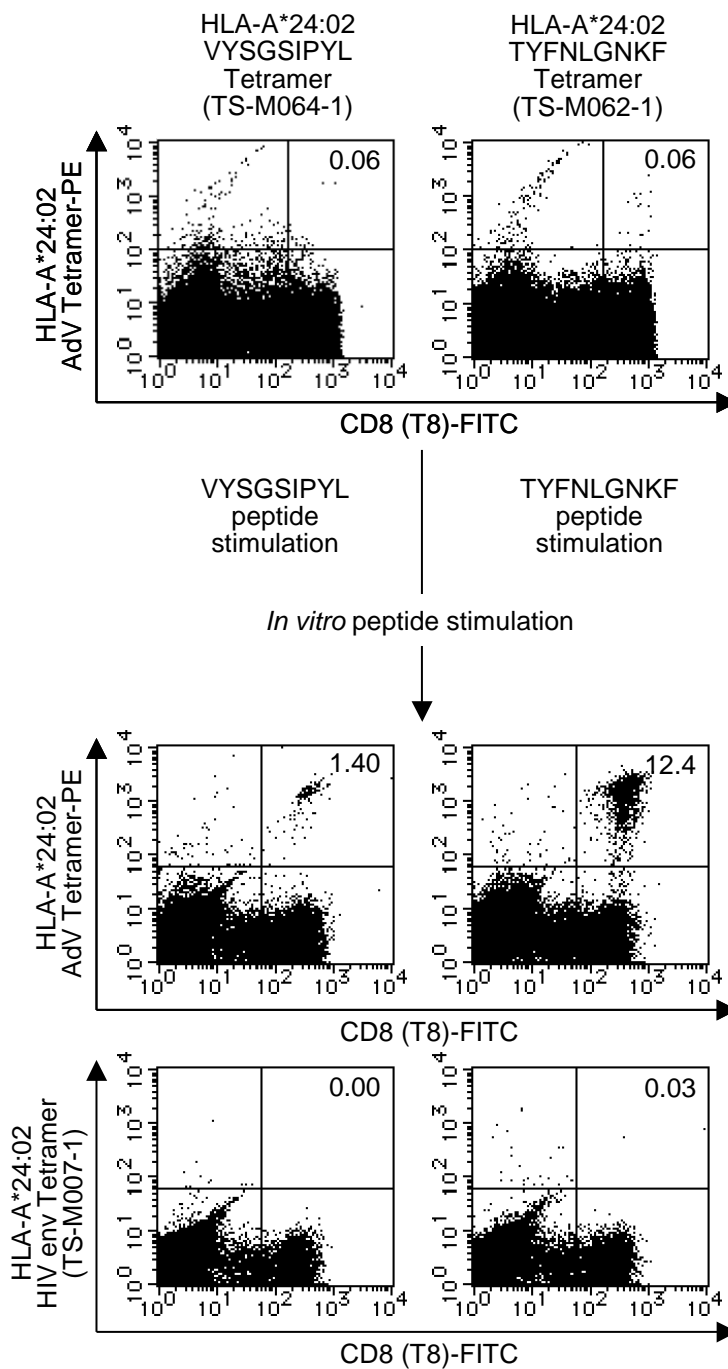
PBMCs from HLA-A\*24:02-positive healthy donors were collected from freshly isolated heparinized peripheral blood according to standard methods.

Aliquots of the PBMCs ( $1 \times 10^6$  cells) were stained with indicated MHC Tetramers (top row) and CD8 antibody.

Another aliquots of PBMCs ( $1-3 \times 10^6$  cells/mL) were incubated in culture tubes in the presence of a synthetic peptide (VYSGSIPYL or TYFNLGNKF, 10  $\mu$ g/mL) and 5% (v/v) autologous plasma. TYFNLGNKF is an another epitope in the AdV11 subgroup B Hexon protein. 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 IL-2 (50 U/mL). After 10 days, aliquots of these cells were stained with MHC Tetramers (as shown in top row), CD8 antibody, and 7-AAD (middle row).

As a control HLA-A\*24:02 tetramer, we used the Negative Tetramer containing the peptide RYL RDQQLL, derived from the human immunodeficiency virus envelope (HIV env) protein (bottom row).

Numbers in the top right quadrants represent the percentage of tetramer-positive cells in the total CD8<sup>high</sup> cells.



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