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

T-Select MHC Tetramer

HLA-A*24:02 survivin-2B Tetramer -AYACNTSTL (50 tests)

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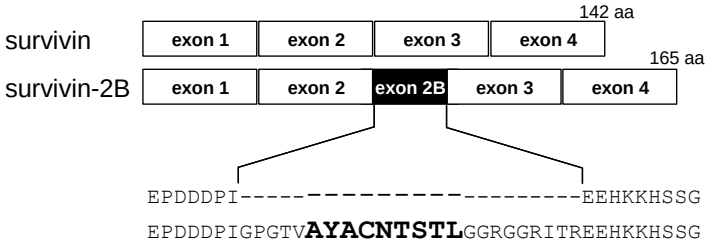
MBL manufactures and distributes these products under license from Beckman Coulter, Inc.. These T-Select MHC Tetramers use patented technology (Japanese patent No. P4780540) of Sapporo Medical University.

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*24:02 and epitope peptide derived from survivin-2B, and it can detect HLA-A*24:02-restricted survivin-2B-specific $CD8^+$ T cells by flow cytometry.

Survivin is a member of the inhibitor of apoptosis protein (IAP) family and is functionally involved in both inhibition of apoptosis and regulation of cell division. There are five different isoforms which include the wild-type survivin, survivin-2B, survivin-3B, survivin-ΔEx3, and survivin-2α. Survivin-2B originates from the insertion of an alternative exon 2B. Dr. Noriyuki Sato and his colleagues at Sapporo Medical University previously reported that survivin-2B was expressed abundantly in various types of tumor tissues and suitable as a target antigen for peptide-based cancer immunotherapy. They have identified an HLA-A24-restricted antigenic peptide, survivin-$2B_{80\text{-}88}$ (AYACNTSTL) derived from exon 2B-encoded region, recognized by $CD8^+$ cytotoxic T lymphocytes (CTL). This CTL epitope has high potency for CTL induction in various cancer patients, including those with breast cancer, colorectal cancer, gastric cancer and oral cancer. A phase II clinical study of survivin-2B peptide vaccination is initiated for patients with advanced or recurrent pancreatic cancer in Japan.



survivin-2B 80-88

HLA Restriction

HLA-A*24:02

Origin and Sequence of CTL Epitope

Survivin-2B (80-88 aa, AYACNTSTL)

Conjugates

TS-M025-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M025-2
Streptavidin-Allophycocyanin (SA-APC)
Excites at 633-635 nm
Emits at 660-680 nm

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.

Storage Conditions

Store at 2 to 8°C. Do not freeze. Minimize exposure to light. The expiration date is indicated on the vial label.

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
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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 These Products

1) Hirohashi Y, *et al*. *Clin Cancer Res* **8**: 1731-1739 (2002)
2) Tsuruma T, *et al*. *J Transl Med* **2**: 19-29 (2004)
3) Idenoue S, *et al*. *Clin Cancer Res* **11**: 1474-1482 (2005)
4) Kurotaki T, *et al*. *J Immunol* **179**: 1803-1813 (2007)
5) Tsuruma T, *et al*. *J Transl Med* **6**: 24-34 (2008)
6) Kutomi G, *et al*. *J Immunol* **183**: 5861-5869 (2009)
7) Miyazaki A, *et al*. *Cancer Sci* **102**: 324-329 (2011)
8) Kameshima H, *et al*. *Cancer Sci* **104**: 124-129 (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 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. 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 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. 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 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

T-Select Human Tetramers

Cancer

TS-M014-1	HLA-A*24:02 WT1 (mutant) Tetramer-CYTWNQMNL-PE
TS-M016-1	HLA-A*02:01 WT1 Tetramer-RMFPNAPYL-PE
TS-M010-1	HLA-A*24:02 hTERT Tetramer-VYGFVRACL-PE
TS-M115-1	HLA-A*02:01 hTERT Tetramer-ILAKFLHWL-PE
TS-M011-1	HLA-A*02:01 NY-ESO-1 Tetramer-SLLMWITQC-PE
TS-M105-1	HLA-A*02:01 NY-ESO-1 C9V Tetramer-SLLMWITQV-PE
TS-M025-1	HLA-A*24:02 survivin-2B Tetramer-AYACNTSTL-PE
TS-M025-2	HLA-A*24:02 survivin-2B Tetramer-AYACNTSTL-APC
TS-0009-1C	HLA-A*02:01 Mart-1 Tetramer-ELAGIGILTV-PE
TS-0013-1C	HLA-A*02:01 gp100 Tetramer-IMDQVPFSV-PE
TS-0014-1C	HLA-A*02:01 gp100 Tetramer-ITDQVPFSV-PE
TS-0015-1C	HLA-A*02:01 Her-2/neu Tetramer-KIFGSLAFL-PE
TS-0016-1	HLA-A*02:01 Her-2/neu Tetramer-RLLQETELV-PE
TS-0017-1	HLA-A*02:01 PR-1 Tetramer-VLQELNVTV-PE
TS-0019-1C	HLA-A*02:01 Tyrosinase Tetramer-YMDGTMSQV-PE
TS-M112-1	HLA-A*24:02 CA9 Tetramer-EYRALQLHL-PE
TS-M114-1	HLA-A*01:01 MAGE-A1 Tetramer-EADPTGHSY-PE
TS-M101-1	HLA-A*02:01 CD33 Tetramer-AIISGDSPV-PE
TS-M102-1	HLA-A*02:01 CD33 A65Y Tetramer-YIISGDSPV-PE
TS-M103-1	HLA-A*02:01 CEA Tetramer-YLSGANLNL-PE
TS-M104-1	HLA-A*02:01 RHAMM Tetramer-ILSLELMKL-PE
TS-M116-1	HLA-A*02:01 $PRAME_{300-309}$ Tetramer-ALYVDSLFFL-PE
TS-M117-1	HLA-A*02:01 $PRAME_{100-108}$ Tetramer-VLDGLDVLL-PE
TS-M118-1	HLA-A*02:01 $PRAME_{425-433}$ Tetramer-SLLQHLIGL-PE
TS-M119-1	HLA-A*02:01 $PRAME_{142-151}$ Tetramer-SLYSFPEPEA-PE
TS-M120-1	HLA-A*02:01 $PSA_{141-150}$ Tetramer-FLTPKKLQCV-PE
TS-M136-1	HLA-A*24:02 PBF A24.2 Tetramer-AYRPVSRNI-PE
TS-M136-2	HLA-A*24:02 PBF A24.2 Tetramer-AYRPVSRNI-APC

Control

TS-M007-1	HLA-A*24:02 Negative Tetramer-RYLRDQQLL-PE
TS-M007-2	HLA-A*24:02 Negative Tetramer-RYLRDQQLL-APC
TS-M007-3	HLA-A*24:02 Negative Tetramer-RYLRDQQLL-FITC

T-Select Peptides

TS-M007-P	HLA-A*24:02 HIV env gp160 peptide
TS-M025-P	HLA-A*24:02 survivin-2B peptide
TS-M136-P	HLA-A*24:02 PBF A24.2 peptide

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

These data were kindly provided by Dr. Toshihiko Torigoe and Dr. Noriyuki Sato, Department of Pathology, Sapporo Medical University.

HLA-A*24:02-positive peripheral blood mononuclear cells (PBMCs) were stimulated with survivin-2B80-88 peptide (MBL, PN TS-M025-P). After 7 to 9 days, cultured cells were stained with a two or three-color MHC-Tetramer staining.

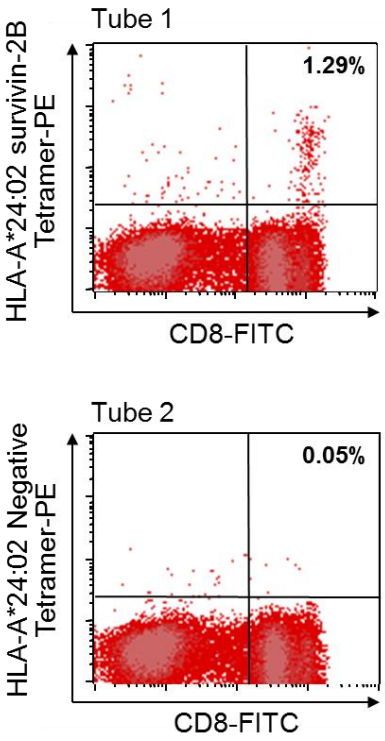
Two-color staining (Results 1)
Tube 1:
HLA-A*24:02 survivin-2B Tetramer-PE/CD8-FITC
Tube 2:
HLA-A*24:02 Negative Tetramer-PE/CD8-FITC

Three-color staining (Results 2)
Tube 3:
HLA-A*24:02 survivin-2B Tetramer-PE
/HLA-A*24:02 Negative Tetramer-FITC
/CD8-PC5

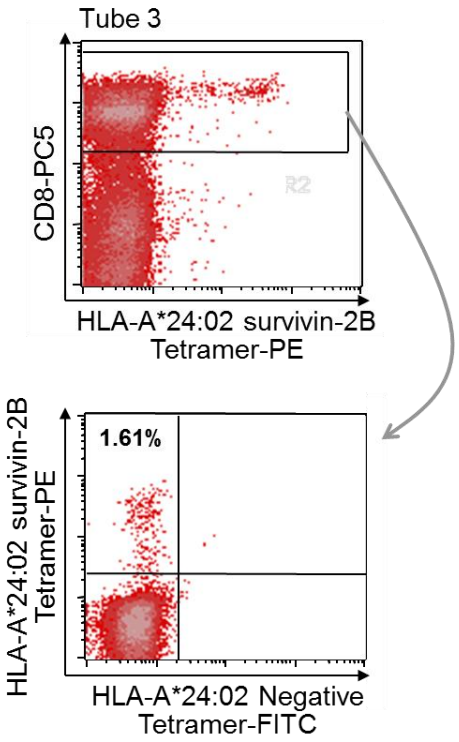
On day 12, Tetramer-positive CTLs were isolated by tetramer-directed flow sorting and cultured under limiting dilution conditions for 2 weeks (day 26, Results 3).

To confirm specificity of MHC Tetramer staining, cells were stained with both specific and negative control MHC Tetramer (MBL, PN TS-M007-1, TS-M007-3) containing the peptide RYLRDQQLL, derived from the human immunodeficiency virus envelope (HIV env) protein.

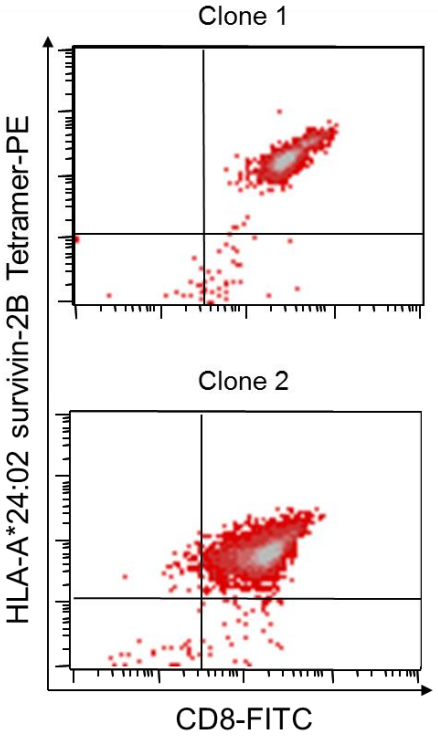
Results 1: Two-color staining



Results 2: Three-color staining



Results 3: Staining of CTL clones



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..
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