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-2B80-88 (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.

HLA Restriction
HLA-A*24:02

Origin and Sequence of CTL Epitope
Survivin-2B (80-88 aa, AYACNTSTL)

Conjugates

<table>
<thead>
<tr>
<th>Conjugate</th>
<th>TS-M025-1</th>
<th>TS-M025-2</th>
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<tbody>
<tr>
<td></td>
<td>Streptavidin-Phycocerythrin (SA-PE)</td>
<td>Streptavidin-Allophycocyanin (SA-APC)</td>
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<tr>
<td>Excites</td>
<td>at 486-580 nm</td>
<td>at 633-635 nm</td>
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<tr>
<td>Emits</td>
<td>at 586-590 nm</td>
<td>at 660-680 nm</td>
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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% NaN3.

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 These Products

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.

References for T-Select MHC Tetramer

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 10^6 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 of 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% NaN₃/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 sources of interference in the analysis of viable cells 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 SFCl21Thy2D3 (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-A2402 WT1 (mutant) Tetramer-CYTWNQMN-LPE
- TS-M016-1 HLA-A2402 WT1 Tetramer-RMFNAPY-LPE
- TS-M010-1 HLA-A2402 HET Tetramer-VYYGFVRACLPE
- TS-M115-1 HLA-A2402 HET Tetramer-ILAKFLH-LPE
- TS-M011-1 HLA-A2402 NY-ESO-1 Tetramer-SLMLWITQGPE
- TS-M105-1 HLA-A2402 NY-ESO-1 C8V Tetramer-SLMLMTQVPE
- TS-M025-1 HLA-A2402 survivin-2B Tetramer-AYACNTSTTLPE
- TS-M025-2 HLA-A2402 survivin-2B Tetramer-AYACNTSTTALPC
- TS-0009-1C HLA-A2402 Mart-1 Tetramer-ELAQGILTVPE
- TS-0013-1C HLA-A2402 gp100 Tetramer-IMDGVPFSVPE
- TS-0014-1C HLA-A2402 gp100 Tetramer-ITDQVFPSV
- TS-0015-1C HLA-A2402 Her-2/neu Tetramer-KIFGSLAFLPE
- TS-0016-1 HLA-A2402 Her-2/neu Tetramer-RLOETELVPE
- TS-0017-1 HLA-A2402 PR-1 Tetramer-VLOELNVTVE
- TS-0019-1C HLA-A2402 Tyrosinase Tetramer-YMDGTMSSOVC
- TS-M112-1 HLA-A2402 C9 Tetramer-EYRALQLHP
- TS-M114-1 HLA-A2402 MAEG-A1 Tetramer-EADPTGHSY
- TS-M101-1 HLA-A2402 CD3 Tetramer-ABSDGPSV
- TS-M102-1 HLA-A2402 CD3 Tetramer-ABSDGPSV
- TS-M103-1 HLA-A2402 CEATetramer-VSLGAGNLPLE
- TS-M104-1 HLA-A2402 RHAMMTetramer-LSLELMKLP
- TS-M116-1 HLA-A2402 FRAME20-90 Tetramer-ALYVDSLFL
- TS-M117-1 HLA-A2402 FRAME20-90 Tetramer-VLODLV
- TS-M118-1 HLA-A2402 FRAME20-90 Tetramer-SLQHILGL
- TS-M119-1 HLA-A2402 FRAME20-90 Tetramer-SLYSFPEPEA
- TS-M120-1 HLA-A2402 PSA141-150 Tetramer-FLTPKJOINV
- TS-M136-1 HLA-A2402 PB2 A242 Tetramer-AIRPVSR
- TS-M136-2 HLA-A2402 PB2 A242 Tetramer-AIRPVSR

Control
- TS-M007-1 HLA-A2402 Negative Tetramer-RLRDKQQLL
- TS-M007-2 HLA-A2402 Negative Tetramer-RLRDKQQLL
- TS-M007-3 HLA-A2402 Negative Tetramer-RLRDKQQLL

T-Select Peptides
- TS-M007-P HLA-A2402 HIV env gp160 peptide
- TS-M025-P HLA-A2402 survivin-2B peptide
- TS-M136-P HLA-A2402 PBF A242 peptide

Others
4844 IMMUNOCYTO CD107a Detection Kit
8223 IMMUNOCYTO IFN-γ ELISPOT Kit
AM1005 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

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 RYLRDQQQL, 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