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

T-Select MHC Tetramer

HLA-DRB1*11:01 TT p2$_{829-844}$ Tetramer -MQYIKANSKFIGITEL (20 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 $CD4^+$ antigen-specific T cells requires cognate recognition of the T cell receptor (TCR) by a class II MHC/peptide complex. This can be done using T-Select MHC class II Tetramers which are composed of four MHC class II 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 $CD4^+$ 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 II HLA-DRB1*11:01 and epitope peptide derived from Tetanus Toxin (TT) p2, and it can detect HLA-DRB1*11:01-restricted TT p2-specific $CD4^+$ T cells by flow cytometry.

Tetanus is caused by the action of a highly potent neurotoxin, tetanospasmin, which is produced during the growth of the anaerobic bacterium *Clostridium tetani* (*Cl. tetani*). Tetanus toxin (TT) is an antigen known to induce strong T cell specific immune responses and is synthesized inside *Cl. tetani* bacterial cells as a single polypeptide chain of 150, 000 molecular weight. Studies of TT-specific $CD4^+$ T-cell epitopes suggest that most individuals exhibit cellular immune responses to a few identical TT epitopes. TT can be inactivated by formaldehyde to yield tetanus toxoid. Tetanus toxoid is used as a vaccine to immunize adults.

A Tetramer, which is constructed with the same allele (HLA-DRB1*11:01) of interest and an irrelevant peptide, may be used as a negative control Tetramer.

HLA Restriction

HLA-DRB1*11:01

Origin and Sequence of This Epitope

Tetanus Toxin (TT p2, 829-844 aa, MQYIKANSKFIGITEL)

References for TT p2$_{829-844}$

1) Demotz S, *et al. J Immunol* **142**: 394 (1989)
2) Okita D, *et al. J Infect Dis* **175**: 382 (1990)
3) Moro M, *et al. BMC Immunol* **6**: 24 (2005)
4) Scriba TJ, *et al. J Immunol* **175**: 6334-6343 (2005)
5) James EA, *et al*. *Int Immunol* **19**: 1291-1301 (2007)
6) Cecconi V, *et al*. *Cytometry A* **73A**: 1010-1018 (2008)

Reagents

200 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.

Conjugates

TS-M812-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M812-2
Streptavidin-Allophycocyanin (SA-APC)
Excites at 633-635 nm
Emits at 660-680 nm

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).

Storage Conditions

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

Usage

This reagent is for use with standard flow cytometry methodologies.

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 acidic 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-CD4-FITC, Beckman Coulter, Inc., PN A07750
- 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-CD4) 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 2×10^7 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. 1×10^6 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-CD4) 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. 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.
E. 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).
F. 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 class II Tetramers

TS-M812-1	HLA-DRB1*11:01 TT $p2_{829-844}$ Tetramer-PE
TS-M812-2	HLA-DRB1*11:01 TT $p2_{829-844}$ Tetramer-APC
TS-M801-1	HLA-DRB1*01:01 human $CLIP_{103-117}$ Tetramer-PE
TS-M801-2	HLA-DRB1*01:01 human $CLIP_{103-117}$ Tetramer-APC
TS-M802-1	HLA-DRB1*01:01 HIV $gag_{295-307}$ Tetramer-PE
TS-M802-2	HLA-DRB1*01:01 HIV $gag_{295-307}$ Tetramer-APC
TS-M803-1	HLA-DRB1*01:01 EBV $EBNA1_{515-527}$ Tetramer-PE
TS-M803-2	HLA-DRB1*01:01 EBV $EBNA1_{515-527}$ Tetramer-APC
TS-M804-1	HLA-DRB1*01:01 Influenza $HA_{306-318}$ Tetramer-PE
TS-M804-2	HLA-DRB1*01:01 Influenza $HA_{306-318}$ Tetramer-APC
TS-M805-1	HLA-DRB1*04:05 human $CLIP_{103-117}$ Tetramer-PE
TS-M805-2	HLA-DRB1*04:05 human $CLIP_{103-117}$ Tetramer-APC
TS-M806-1	HLA-DRB1*04:05 Influenza $HA_{306-318}$ Tetramer-PE
TS-M806-2	HLA-DRB1*04:05 Influenza $HA_{306-318}$ Tetramer-APC
TS-M807-1	HLA-DRB1*11:01 human $CLIP_{103-117}$ Tetramer-PE
TS-M807-2	HLA-DRB1*11:01 human $CLIP_{103-117}$ Tetramer-APC
TS-M808-1	HLA-DRB1*11:01 Influenza $HA_{306-318}$ Tetramer-PE
TS-M808-2	HLA-DRB1*11:01 Influenza $HA_{306-318}$ Tetramer-APC
TS-M809-1	HLA-DRB1*04:01 human $CLIP_{103-117}$ Tetramer-PE
TS-M809-2	HLA-DRB1*04:01 human $CLIP_{103-117}$ Tetramer-APC
TS-M810-1	HLA-DRB1*04:01 Influenza $HA_{306-318}$ Tetramer-PE
TS-M810-2	HLA-DRB1*04:01 Influenza $HA_{306-318}$ Tetramer-APC
TS-M811-1	HLA-DRB1*04:01 $GAD65_{555-567}$ Tetramer-PE
TS-M811-2	HLA-DRB1*04:01 $GAD65_{555-567}$ Tetramer-APC
TS-M815-1	HLA-DRB1*01:01 HTLV-1 $Tax_{155-167}$ Tetramer-PE
TS-M815-2	HLA-DRB1*01:01 HTLV-1 $Tax_{155-167}$ Tetramer-APC
TS-M816-1	HLA-DRB1*15:01 human $CLIP_{103-117}$ Tetramer-PE
TS-M816-2	HLA-DRB1*15:01 human $CLIP_{103-117}$ Tetramer-APC
TS-M817-1	HLA-DRB1*15:02 human $CLIP_{103-117}$ Tetramer-PE
TS-M817-2	HLA-DRB1*15:02 human $CLIP_{103-117}$ Tetramer-APC

T-Select Mouse class II Tetramers

TS-M703-1	I-A^d $OVA_{323-339}$ Tetramer-PE
TS-M704-1	I-A^b MOG_{35-55} Tetramer-PE
TS-M705-1	I-A^b $FMLV_{123-141}$ Tetramer-PE
TS-M706-1	I-A^b $E\alpha_{52-68}$ Tetramer-PE
TS-M707-1	I-A^b ESAT-6_{1-20} Tetramer-PE
TS-M710-1	I-A^b $OVA_{323-339}$ Tetramer-PE
TS-M715-1	I-A^b human $CLIP_{103-117}$ Tetramer-PE
TS-M716-1	I-A^b Influenza $NP_{311-325}$ Tetramer-PE
TS-M720-1	I-A^d human $CLIP_{103-117}$ Tetramer-PE
TS-M721-1	I-A^b *L. monocytogenes* $LLO_{190-201}$ Tetramer-PE
TS-M722-1	I-A^b mouse 2W1S Tetramer-PE
TS-M724-1	I-A^b LCMV $GP_{126-140}$ Tetramer-PE

T-Select Peptides

TS-M812-P	HLA-DRB1*11:01 TT $p2_{829-844}$ peptide
TS-M801-P	HLA-DRB1*01:01 human $CLIP_{103-117}$ peptide
TS-M802-P	HLA-DRB1*01:01 HIV $gag_{295-307}$ peptide
TS-M803-P	HLA-DRB1*01:01 EBV $EBNA1_{515-527}$ peptide
TS-M804-P	HLA-DRB1*01:01 Influenza $HA_{306-318}$ peptide
TS-M811-P	HLA-DRB1*04:01 $GAD65_{555-567}$ peptide
TS-M815-P	HLA-DRB1*01:01 HTLV-1 $Tax_{155-167}$ peptide
TS-M701-P	I-A^b HBc helper peptide
TS-M702-P	I-A^d Tetanus toxin p30 helper peptide
TS-M703-P	I-A^b/I-A^d OVA helper peptide
TS-M704-P	I-A^b MOG_{35-55} peptide
TS-M707-P	I-A^b ESAT-6_{1-20} peptide
TS-M708-P	I-A^k HEL peptide
TS-M716-P	I-A^b Influenza $NP_{311-325}$ peptide
TS-M721-P	I-A^b *L. monocytogenes* $LLO_{190-201}$ peptide
TS-M722-P	I-A^b mouse 2W1S peptide
TS-M724-P	I-A^b LCMV $GP_{126-140}$ peptide

Kit

AM-1005M	IMMUNOCYTO Cytotoxicity Detection Kit
4844	IMMUNOCYTO CD107a Detection Kit

Others

A07750	Anti-CD4 (Human) mAb-FITC
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-DRB1*11:01-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 - 80 °C.

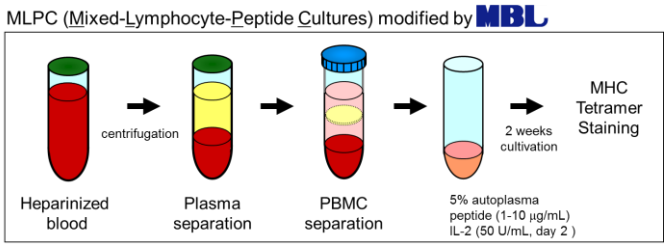
Aliquots of the PBMCs (1x10^6 cells) were stained with TT p2 Tetramer (MBL, PN TS-M812-1) and CD4 antibody (day 0). Another aliquots of PBMCs (1-3 x 10^6 cells/mL) were incubated in culture tubes (Round-Bottom Tube, Falcon®, PN 352059) in the presence of a synthetic peptide (10 μg/mL of MQYIKANSKFIGITEL, TS-M812-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). After 14 days, aliquots of these cells were stained with these MHC Tetramers, CD4 antibody, and 7-AAD (after MLPC).

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). Data were analyzed by double gating on the lymphocyte and viable cell population (R1 and R2). Numbers in the top right quadrants represent the percentage of MHC Tetramer-positive cells in the total $CD4^+$ cells.

To confirm specificity of MHC Tetramer staining, cells were stained with specific and negative control MHC Tetramer. DRB1*11:01 human $CLIP_{103-117}$ Tetramer (MBL, PN TS-M807-1) was used as a negative control, containing the peptide PVSKMRMATPLLMQA, CLIP.

Results showed that HLA-DRB1*11:01-restricited TT $p2_{829-844}$ (MQYIKANSKFIGITEL)-specific $CD4^+$ T cells were detectable in the peptide stimulated PBMCs.



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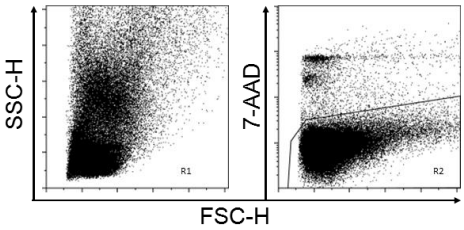

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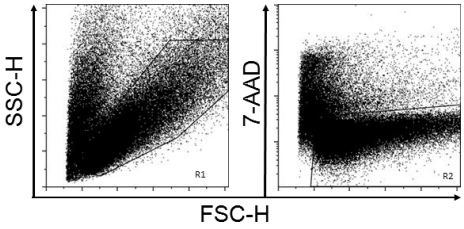

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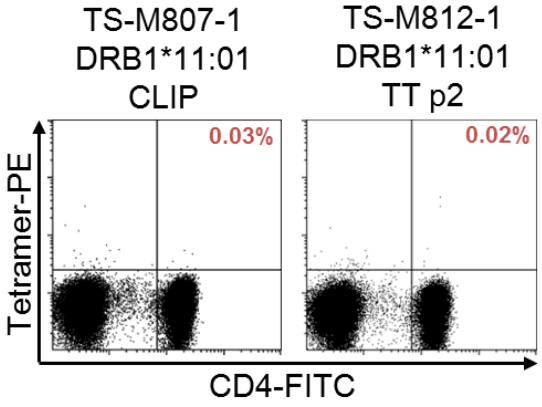
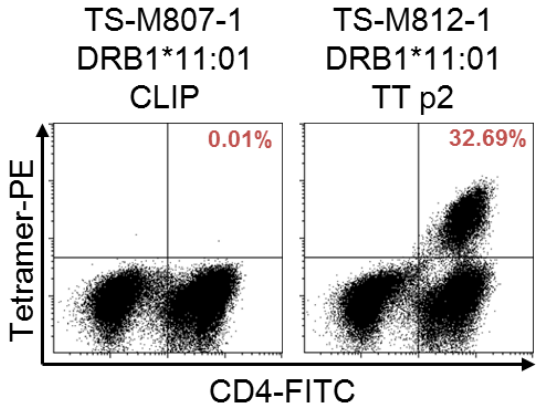


+ TT p2 peptide
(final conc. 10 μg/mL)

<after MLPC>



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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