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

T-Select MHC Tetramer

HLA-DRB1*15:02 human CLIP$_{103-117}$ Tetramer -PVSKMRMATPLLMQA (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*15:02 and epitope peptide CLIP derived from human class II associated Invariant chain (Ii).

CLIP is the part of the Ii, which is a chaperone molecule. In antigen presenting cells, MHC class II molecules assemble in the ER with Ii. The CLIP segment of Ii binds the peptide binding groove of MHC class II molecules to protect the hydrophobic binding site. Following transport to the endosomal/lysosomal peptide-loading compartment, Ii is proteolytically cleaved, and the remaining CLIP peptide is exchanged with other peptides in a reaction catalyzed by HLA-DM.

This Tetramer can be used as a negative control Tetramer to different epitopes you are interested in of the same allele (HLA-DRB1*15:02).

HLA Restriction

HLA-DRB1*15:02

Origin and Sequence of This Epitope

Human class II associated Invariant chain peptide CLIP (103-117 aa, PVSKMRMATPLLMQA)

References for These Products

1) Paul A, *et al. Nature* **345**: 615-618 (1990)
2) Victor S, *et al. Nature* **375**: 802-806 (1995)
3) Sebastian A, *et al. J Exp Med* **181**: 1729-1741 (1995)
4) Lisa K, *et al. J Cell* **82**: 155-165 (1995)
5) Felix B, *et al. PNAS* **98**: 12168-12173 (2001)
6) Cheryl LD, *et al. J Clin Invest* **112**: 831-842 (2003)
7) Gerald TN, *et al. J Immunol* **188**: 2477-2482 (2012)

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 0.1% Proclin™ 150.

Conjugates

TC-M817-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TC-M817-2
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 (PE Tetramer), or light blue (APC Tetramer).

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. Specimens, samples and material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
2. Never pipette by mouth and avoid contact of samples with skin and mucous membranes.
3. Minimize exposure of reagent to light during storage or incubation.
4. Avoid microbial contamination of reagent or erroneous results may occur.
5. 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. Analyze prepared samples by flow cytometry. If necessary, store the samples at 2-8°C protected from light for a maximum of 24 hours prior to analysis.

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-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. Analyze prepared samples by flow cytometry. If necessary, store the samples at 2-8°C protected from light for a maximum of 24 hours prior to analysis.

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

TC-M801-1 HLA-DRB1*01:01 human $CLIP_{103-117}$ Tetramer-PE
TC-M801-2 HLA-DRB1*01:01 human $CLIP_{103-117}$ Tetramer-APC
TC-M802-1 HLA-DRB1*01:01 HIV $gag_{295-307}$ Tetramer-PE
TC-M802-2 HLA-DRB1*01:01 HIV $gag_{295-307}$ Tetramer-APC
TC-M803-1 HLA-DRB1*01:01 EBV $EBNA1_{515-527}$ Tetramer-PE
TC-M803-2 HLA-DRB1*01:01 EBV $EBNA1_{515-527}$ Tetramer-APC
TC-M804-1 HLA-DRB1*01:01 Influenza $HA_{306-318}$ Tetramer-PE
TC-M804-2 HLA-DRB1*01:01 Influenza $HA_{306-318}$ Tetramer-APC
TC-M805-1 HLA-DRB1*04:05 human $CLIP_{103-117}$ Tetramer-PE
TC-M805-2 HLA-DRB1*04:05 human $CLIP_{103-117}$ Tetramer-APC
TC-M806-1 HLA-DRB1*04:05 Influenza $HA_{306-318}$ Tetramer-PE
TC-M806-2 HLA-DRB1*04:05 Influenza $HA_{306-318}$ Tetramer-APC
TC-M807-1 HLA-DRB1*11:01 human $CLIP_{103-117}$ Tetramer-PE
TC-M807-2 HLA-DRB1*11:01 human $CLIP_{103-117}$ Tetramer-APC
TC-M808-1 HLA-DRB1*11:01 Influenza $HA_{306-318}$ Tetramer-PE
TC-M808-2 HLA-DRB1*11:01 Influenza $HA_{306-318}$ Tetramer-APC
TC-M809-1 HLA-DRB1*04:01 human $CLIP_{103-117}$ Tetramer-PE
TC-M809-2 HLA-DRB1*04:01 human $CLIP_{103-117}$ Tetramer-APC
TC-M810-1 HLA-DRB1*04:01 Influenza $HA_{306-318}$ Tetramer-PE
TC-M810-2 HLA-DRB1*04:01 Influenza $HA_{306-318}$ Tetramer-APC
TC-M811-1 HLA-DRB1*04:01 $GAD65_{555-567}$ Tetramer-PE
TC-M811-2 HLA-DRB1*04:01 $GAD65_{555-567}$ Tetramer-APC
TC-M812-1 HLA-DRB1*11:01 TT $p2_{829-844}$ Tetramer-PE
TC-M812-2 HLA-DRB1*11:01 TT $p2_{829-844}$ Tetramer-APC
TC-M815-1 HLA-DRB1*01:01 HTLV-1 $Tax_{155-167}$ Tetramer-PE
TC-M815-2 HLA-DRB1*01:01 HTLV-1 $Tax_{155-167}$ Tetramer-APC
TC-M816-1 HLA-DRB1*15:01 human $CLIP_{103-117}$ Tetramer-PE
TC-M816-2 HLA-DRB1*15:01 human $CLIP_{103-117}$ Tetramer-APC
TC-M817-1 HLA-DRB1*15:02 human $CLIP_{103-117}$ Tetramer-PE
TC-M817-2 HLA-DRB1*15:02 human $CLIP_{103-117}$ Tetramer-APC
TC-M703-2 I-A^d $OVA_{323-339}$ Tetramer-APC
TC-M704-1 I-A^b MOG_{35-55} Tetramer-PE
TC-M704-2 I-A^b MOG_{35-55} Tetramer-APC
TC-M707-1 I-A^b ESAT-6_{1-20} Tetramer-PE
TC-M710-1 I-A^b $OVA_{323-339}$ Tetramer-PE
TC-M710-2 I-A^b $OVA_{323-339}$ Tetramer-APC
TC-M715-1 I-A^b human $CLIP_{103-117}$ Tetramer-PE
TC-M715-2 I-A^b human $CLIP_{103-117}$ Tetramer-APC
TC-M716-1 I-A^b Influenza $NP_{311-325}$ Tetramer-PE
TC-M716-2 I-A^b Influenza $NP_{311-325}$ Tetramer-APC
TC-M720-1 I-A^d human $CLIP_{103-117}$ Tetramer-PE
TC-M720-2 I-A^d human $CLIP_{103-117}$ Tetramer-APC
TC-M721-1 I-A^b *L. monocytogenes* $LLO_{190-201}$ Tetramer-PE
TC-M721-2 I-A^b *L. monocytogenes* $LLO_{190-201}$ Tetramer-APC
TC-M722-1 I-A^b mouse 2W1S Tetramer-PE
TC-M722-2 I-A^b mouse 2W1S Tetramer-APC
TC-M724-1 I-A^b LCMV $GP_{126-140}$ Tetramer-PE
TC-M724-2 I-A^b LCMV $GP_{126-140}$ Tetramer-APC

Kit

TS-7300-K1 QuickSwitch Quant HLA-A*02:01 Tetramer Kit-PE
TS-7301-K1 QuickSwitch HLA-A*02:01 Tetramer Kit-PE

Others

MTG-001 Clear Back (Human FcR blocking reagent)

Please check our website (https://www.mbl-chinawide.cn) for up-to-date information on products and custom MHC Tetramers.