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

T-Select MHC Class I Mouse Tetramer

Allele and Peptide Specificity

The T-Select MHC Class I Mouse Tetramers recognize murine $CD8^+$ T cells which are specific for a particular peptide in combination with the H-2 murine alleles.

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 class I MHC Tetramers which are composed of a complex of four H-2 MHC class I molecules each bound to the specific peptide[1), 2)] 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 all specific $CD8^+$ T cells regardless of functional status. Measurements may be performed in whole blood or isolated lymphocyte/splenocyte or thymocyte cell preparations[3)]. 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.

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.

Conjugates

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

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

- Streptavidin-Fluorescein Isothiocyanate (SA-FITC)
  Excites at 465-495 nm
  Emits at 515-555 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.
If the expiration date is not indicated, T-Select MHC Tetramers are stable for 90 days from the date of purchase. Stability data are not available for custom T-Select MHC Tetramers.

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), light blue (SA-APC), or light yellow liquid (SA-FITC).

Reagent Preparation

No preparation is necessary. These T-Select MHC Tetramer reagents are used directly from the vial after a brief vortex on low setting.

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 pipet 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.

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp

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-mouse CD8-FITC (clone KT15), MBL, PN D271-4
- Anti-mouse CD8-Alexa Fluor® 647 (clone KT15), MBL, PN D271-A64
- 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 venous blood specimen according to established protocol into a blood collection tube using an appropriate anti-coagulant. If the mouse line that is being used is transgenic and the T cell receptor is specific for the peptide, 100 μL of whole blood should be adequate. If the blood specimen is not being derived from a transgenic line, you may require more than 100 μL in order to perform the rare event analysis.
2. To each 12 x 75 mm test tube add 10 μL of T-Select MHC Tetramer.
3. Add 100 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 Cell Preparations and Cell Suspensions

1. Collect lymph node, spleen or thymus and prepare a single-cell suspension according to an established protocol. 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 of cell suspension into each test tube (e.g. 1 x 10^6 cells per tube).
4. Incubate for 5 minutes at room temperature (15-25°C).
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.
   If red blood cell lysis is necessary, proceed to step 8-9 in the **Procedure for Whole Blood** section. If red blood cell lysis is not necessary, continue to step 9 below.
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% paraformaldehyde or formalin.
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 cell cultivation 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, which is constructed with the same allele of interest and an irrelevant peptide, may also be used as a negative control.
D. We recommend the use of the CD8 antibody (clone KT15), because some CD8 antibodies inhibit Tetramer–specific binding to TCR.

E. In the case of OT-I TCR transgenic mice, it is necessary to perform a cross-titration experiment with the Tetramer and the CD8 antibody (clone KT15) to determine the optimal concentration of both reagents.
F. 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.
G. 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).
H. Cells do not require fixation prior to analysis if the stained cells are analyzed by flow cytometry within several hours.

Selected References

1) Altman JD, Moss PH, Goulder PJR, Barouch DH, McHeyzer W, Bell JI, McMichael AJ, and Davis MM. 1996. Phenotypic Analysis of Antigen-Specific T Lymphocytes. Science 274:94-96.
2) McMichael AJ, and O 'Callaghan CA. 1998. A New Look at T Cells. J. Exp. Med. 187:1367-1371.
3) Skinner PJ, Daniels MA, Schmidt CS, Jameson SC, and Haase AT. 2000. In Situ Tetramer Staining of Antigen-Specific T Cells in Tissues. J. Immunol. 165:613-617.
4) Nugent CT, Morgan DJ, Biggs JA, Ko A, Pilip IM, Pamer EG and Sherman LA. 2000. Characterization of $CD8^+$ T Lymphocytes That Persist After Peripheral Tolerance to a Self Antigen Expressed in the Pancreas. J. Immunol. 164:191-200.

Related Products

T-Select Mouse Tetramers

Cancer

TS-5004-1C	H-2K^b TRP2 Tetramer-SVYDFFVWL-PE
TS-M504-1	H-2D^b WT1 Tetramer-RMFPNAPYL-PE
TS-M505-1	H-2D^b human gp100 Tetramer-KVPRNQDWL-PE
TS-M518-1	H-2D^b CEA Tetramer-EAQNTTYL-PE
TS-M519-1	H-2L^d P815 Tetramer-LPYLGWLVF-PE
TS-M526-1	H-2K^d HER2 Tetramer-TYLPTNASL-PE
TS-M544-1	H-2K^d JAK1 Tetramer-SYFPEITHI-PE
TS-M545-1	H-2K^d Erk2 K136Q Tetramer-QYIHSANVL-PE
TS-M546-1	H-2D^b gp100 Tetramer-EGSRNQDWL-PE
TS-M558-1	H-2K^b MAGE-AX$_{169-176}$ Tetramer-LGITYDGM-PE
TS-M559-1	H-2K^b MAGE-A5 Tetramer-HNTQYCNL-PE

Influenza

TS-M502-1	H-2D^b Influenza NP Tetramer-ASNENMDTM-PE
TS-M508-1	H-2D^b Influenza NP Tetramer-ASNENMETM-PE
TS-M527-1	H-2D^b Influenza NP Tetramer-ASNENMDAM-PE
TS-M528-1	H-2D^b Influenza PA Tetramer-SSLENFRAYV-PE
TS-M533-1	H-2K^b Influenza PB1 Tetramer-SSYRRPVGI-PE
TS-M520-1	H-2K^d Influenza HA Tetramer-IYSTVASSL-PE
TS-M535-1	H-2K^d Influenza HA Tetramer-LYQNVGTYV-PE
TS-M534-1	H-2K^d Influenza NP Tetramer-TYQRTRALV-PE

LCMV

TS-5002-1C	H-2D^b LCMV gp33 Tetramer-KAVYNFATC-PE
TS-5009-1	H-2D^b LCMV gp$_{276-286}$ Tetramer-SGVENPGGYCL-PE
TS-5010-1C	H-2K^b LCMV gp$_{34-41}$ Tetramer-AVYNFATC-PE
TS-5011-1	H-2K^b LCMV gp$_{34-43}$ Tetramer-AVYNFATCGI-PE
TS-5012-1	H-2K^b LCMV gp$_{118-125}$ Tetramer-ISHNFCNL-PE
TS-5014-1	H-2K^b LCMV L protein Tetramer-LEYDFNKL-PE
TS-5015-1	H-2K^b LCMV NP$_{205-212}$ Tetramer-YTVKYPNL-PE
TS-M512-1	H-2D^b LCMV gp33 (C9M) Tetramer-KAVYNFATM-PE
TS-M513-1	H-2D^b LCMV NP396 Tetramer-FQPQNGQFI-PE
TS-M514-1	H-2L^d LCMV NP118 Tetramer-RPQASGVYM-PE

HIV

TS-M516-1	H-2D^d HIV P18-I10 Tetramer-RGPGRAFVTI-PE
TS-5007-1	H-2K^b HIV gag Tetramer-AMQMLKETI-PE
TS-M536-1	H-2D^d HIV env Tetramer-IGPGRAFYA-PE

RSV

TS-M506-1	H-2K^d RSV Tetramer-SYIGSINNI-PE
TS-M555-1	H-2K^d RSV F glycoprotein Tetramer-KYKNAVTEL-PE
TS-5018-1C	H-2D^b RSV Tetramer-NAITNAKII-PE

SV40

TS-M539-1	H-2D^b SV40 large T Ag$_{206-215}$ Tetramer-SAINNYAQKL-PE
TS-M540-1	H-2D^b SV40 large T Ag$_{489-497}$ Tetramer-QGINNLDNL-PE

MuLV

TS-M507-1	H-2K^b MuLV p15E Tetramer-KSPWFTTL-PE
TS-M521-1	H-2L^d MuLV gp70 Tetramer-SPSYVYHQF-PE

Virus

TS-M509-1	H-2K^b SeV Tetramer-FAPGNYPAL-PE
TS-M510-1	H-2L^d MCMV IE1 Tetramer-YPHFMPTNL-PE
TS-M522-1	H-2L^d HBsAg Tetramer-IPQSLDSWWTSL-PE
TS-M523-1	H-2K^b HSV-1 gB Tetramer-SSIEFARL-PE
TS-M529-1	H-2K^b VSV NP Tetramer-RGYVYQGL-PE
TS-M530-1	H-2D^k polyomavirus MT Tetramer-RRLGRTLLL-PE
TS-M531-1	H-2D^k HTLV-1 Tax$_{38-46}$ Tetramer-ARLHRHALL-PE
TS-M532-1	H-2D^b HCV NS3$_{1629-1637}$ Tetramer-GAVQNEVTL-PE
TS-M537-1	H-2K^b HBV core Tetramer-MGLKFRQL-PE
TS-M538-1	H-2K^b VACV B8R Tetramer-TSYKFESV-PE
TS-5008-1C	H-2D^b HPV16 E7 Tetramer-RAHYNIVTF-PE
TS-5016-1	H-2D^b MoMSV Tetramer-(Abu)(Abu)L(Abu)LTVFL-PE
TS-5017-1C	H-2D^b SIV gag Tetramer-AAVKNWMTQTL-PE

OVA

TS-5001-1C	H-2K^b OVA Tetramer-SIINFEKL-PE
TS-M541-1	H-2K^b OVA E1 Tetramer-EIINFEKL-PE
TS-M542-1	H-2K^b OVA G4 Tetramer-SIIGFEKL-PE
TS-M543-1	H-2K^b OVA Q4H7 Tetramer-SIIQFEHL-PE

Foreign antigen

TS-M525-1	H-2K^d EGFP Tetramer-HYLSTQSAL-PE
TS-M501-1	H-2K^b β-galactosidase Tetramer-DAPIYTNV-PE
TS-M511-1	H-2L^d β-galactosidase Tetramer-TPHPARIGL-PE

Bacteria

TS-M503-1 H-2K^d Listeria LLO Tetramer-GYKDGNEYI-PE
TS-M515-1 H-2K^d malaria Tetramer-SYIPSAEKI-PE
TS-M547-1 H-2K^d Plasmodium CSP Tetramer-SYVPSAEQI-PE
TS-M548-1 H-2D^b Chlamydia CrpA Tetramer-ASFVNPIYL-PE
TS-M560-1 H-2K^b TSKB20 Tetramer-ANYKFTLV-PE

Mycobacterium tuberculosis

TS-M517-1 H-2D^d BCG MPT51 Tetramer-GGPHAVYLL-PE
TS-M549-1 H-2D^b Mtb32a Tetramer-GAPINSATAM-PE
TS-M550-1 H-2K^b TB10.4 Tetramer-IMYNYPAM-PE

Others

TS-M524-1 H-2D^b HY Uty Tetramer-WMHHNMDLI-PE
TS-M551-1 H-2K^b HA-60 Tetramer-LTFNYRNL-PE
TS-M552-1 H-2K^d IGRP Tetramer-VYLKTNVFL-PE
TS-M553-1 H-2K^d NRP-V7 Tetramer-KYNKANVFL-PE
TS-M554-1 H-2K^d InsB Tetramer-LYLVCGERL-PE
TS-M557-1 H-2D^b MimA2 Tetramer-YAIENYLEL-PE

MHC Class II Tetramers

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-M710-2 I-A^b $OVA_{323-339}$ Tetramer-APC

CD1d Tetramers

TS-MCD-1 Mouse CD1d Tetramer-PE
TS-MCD-2 Mouse CD1d Tetramer-APC

T-Select Peptides

TS-5001-P H-2K^b OVA peptide
TS-5002-P H-2D^b LCMV gp33 peptide
TS-5004-P H-2K^b TRP-2 peptide
TS-5008-P H-2D^b HPV16 E7 peptide
TS-M501-P H-2K^b β-galactosidase peptide
TS-M502-P H-2D^b Influenza NP peptide
TS-M508-P H-2D^b Influenza NP peptide
TS-M527-P H-2D^b Influenza NP peptide
TS-M520-P H-2K^d Influenza HA peptide
TS-M528-P H-2D^b Influenza PA peptide
TS-M503-P H-2K^d Listeria LLO peptide
TS-M505-P H-2D^b human gp100 peptide
TS-M506-P H-2K^d RSV peptide
TS-M507-P H-2K^b MuLV p15E peptide
TS-M521-P H-2L^d MuLV gp70 peptide
TS-M509-P H-2K^b SeV peptide
TS-M510-P H-2L^d MCMV IE1 peptide
TS-M511-P H-2L^d β-galactosidase peptide
TS-M512-P H-2D^b LCMV gp33 (C9M) peptide
TS-M513-P H-2D^b LCMV NP396 peptide
TS-M514-P H-2L^d LCMV NP118 peptide
TS-M515-P H-2K^d malaria peptide
TS-M516-P H-2D^d HIV P18-I10 peptide
TS-M517-P H-2D^d BCG MPT51 peptide
TS-M518-P H-2D^b CEA peptide
TS-M519-P H-2L^d P815 peptide
TS-M522-P H-2L^d HBsAg peptide
TS-M523-P H-2K^b HSV-1 gB peptide
TS-M524-P H-2D^b HY Uty peptide
TS-M525-P H-2K^d EGFP peptide
TS-M526-P H-2K^d HER2 peptide
TS-M529-P H-2K^b VSV NP peptide
TS-M530-P H-2D^k polyomavirus MT peptide
TS-M531-P H-2D^k HTLV-1 Tax_{38-46} peptide
TS-M008-P H-2K^b SIY 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

Kit

AM-1005 IMMUNOCYTO Cytotoxity Detection Kit

Others

D341-4 mouse CD4-FITC (GK1.5)
D271-4 mouse CD8-FITC (KT15)
D271-A64 mouse CD8-Alexa Fluor® 647 (KT15)
K0221-3 anti-mouse TCR DO11.10 (KJ1.26)
K0221-5 anti-mouse TCR DO11.10-PE (KJ1.26)
K0222-3 anti-mouse TCR 3DT-52.5 (KJ12.98)
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.