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 function. 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

T-Select MHC Class I Mouse Tetramer - 50 tests
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.
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Materials Required But Not Supplied

- 12x75 mm polypropylene test tubes
- Transfer pipettes
- Pipettors and disposable pipette tips
- Vortex
- Centrifuge capable of 150 x g or 400 x g
- Aspirator
- PBS
- iTAg™ MHC Tetramer Lyse Reagent, Beckman Coulter, Inc., PN T08002
- iTAg™ MHC Tetramer Fixative Reagent, Beckman Coulter, Inc., PN T08003
- Anti-CD8-FITC (clone KT15), MBL, PN D271-4
- 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 12x75 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 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 1 mL of Lyse Reagent supplemented with 25 μL Fixative Reagent per tube.
9. Vortex for 5 seconds immediately after the addition of the Lyse/Fixative solution per tube.
10. Incubate for a minimum of 10 minutes at room temperature protected from light.
11. Centrifuge tubes at 150 x g for 5 minutes.
12. Aspirate or decant the supernatant.
13. Add 3 mL of PBS and centrifuge tubes at 150 x g for 5 minutes.
14. Aspirate or decant the supernatant.
15. Resuspend the pellet in 500 μL of PBS with 0.5% paraformaldehyde or formalin.
16. Store at 4°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. To each 12x75 mm test tube add 10 μL of Clear Back (human FcR blocking reagent, MBL PN MTG-001).
3. Add 50 μL cell suspension 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 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 8-16 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 at 4°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. In an experiment where cells are stained with T-Select MHC Tetramer and antibodies, Clear Back (human FcR blocking reagent) may effectively block non-specific binding caused by macrophages or endocytosis, resulting in clear staining. Please refer to the data sheet (MBL PN MTG-001) for details.
B. A Tetramer, which is constructed with the same allele of interest and an irrelevant peptide, may also be used as a negative control.
C. We recommend the use of the CD8 antibody (clone KT15), because some CD8 antibodies inhibit Tetramer–specific binding to TCR.
D. 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.
E. To reduce contamination of unlysed or nucleated red blood cells in the gate, we recommend the use of CD45 antibody and gating of the lymphocyte population.
F. Apoptotic, necrotic, and/or damaged cells are sources of interference in the analysis of viable cells by flow cytometry. Non-viable cells should be evaluated and discriminated following 7-AAD-positive labeling when viable cells remain unstained (negative).
G. The cells do not need to be fixed before analysis if 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-5004-2C	H-2K^b TRP2 Tetramer-SVYDFFVWL-APC
TS-M504-1	H-2D^b WT1 Tetramer-RMFPNAPYL-PE
TS-M504-2	H-2D^b WT1 Tetramer-RMFPNAPYL-APC
TS-M505-1	H-2D^b human gp100 Tetramer-KVPRNQDWL-PE
TS-M505-2	H-2D^b human gp100 Tetramer-KVPRNQDWL-APC
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

Virus

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-M520-1	H-2K^d Influenza HA Tetramer-IYSTVASSL-PE
TS-5002-1	H-2D^b LCMV gp33 Tetramer-KAVYNFATC-PE
TS-5002-2	H-2D^b LCMV gp33 Tetramer-KAVYNFATC-APC
TS-5009-1	H-2D^b LCMV gp276-286 Tetramer-SGVENPGGYCL-PE
TS-5009-2	H-2D^b LCMV gp276-286 Tetramer-SGVENPGGYCL-APC
TS-5010-1	H-2K^b LCMV gp34-41 Tetramer-AVYNFATC-PE
TS-5010-2	H-2K^b LCMV gp34-41 Tetramer-AVYNFATC-APC
TS-5011-1	H-2K^b LCMV gp34-43 Tetramer-AVYNFATCGI-PE
TS-5011-2	H-2K^b LCMV gp34-43 Tetramer-AVYNFATCGI-APC
TS-5012-1	H-2K^b LCMV gp118-125 Tetramer-ISHNFCNL-PE
TS-5012-2	H-2K^b LCMV gp118-125 Tetramer-ISHNFCNL-APC
TS-5014-1	H-2K^b LCMV L protein Tetramer-LEYDFNKL-PE
TS-5014-2	H-2K^b LCMV L protein Tetramer-LEYDFNKL-APC
TS-5015-1	H-2K^b LCMV NP205-212 Tetramer-YTVKYPNL-PE
TS-5015-2	H-2K^b LCMV NP205-212 Tetramer-YTVKYPNL-APC
TS-M512-1	H-2D^b LCMV gp33 (C9M) Tetramer-KAVYNFATM-PE
TS-M513-1	H-2D^b LCMV NP396 Tetramer-FQPQNGQFI-PE
TS-M513-2	H-2D^b LCMV NP396 Tetramer-FQPQNGQFI-APC
TS-M514-1	H-2L^d LCMV NP118 Tetramer-RPQASGVYM-PE
TS-M516-1	H-2D^d HIV P18-I10 Tetramer-RGPGRAFVTI-PE
TS-5007-1	H-2K^b HIV gag Tetramer-AMQMLKETI-PE
TS-5007-2	H-2K^b HIV gag Tetramer-AMQMLKETI-APC
TS-5008-1C	H-2D^b HPV16 E7 Tetramer-RAHYNIVTF-PE
TS-5008-2C	H-2D^b HPV16 E7 Tetramer-RAHYNIVTF-APC
TS-M506-1	H-2K^d RSV Tetramer-SYIGSINNI-PE
TS-M506-2	H-2K^d RSV Tetramer-SYIGSINNI-APC
TS-5018-1	H-2D^b RSV Tetramer-NAITNAKII-PE
TS-5018-2	H-2D^b RSV Tetramer-NAITNAKII-APC
TS-M507-1	H-2K^b MuLV p15E Tetramer-KSPWFTTL-PE
TS-M521-1	H-2L^d MuLV gp70 Tetramer-SPSYVYHQF-PE
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 Tax38-46 Tetramer-ARLHRHALL-PE
TS-5016-1	H-2D^{b} MoMSV Tetramer-(Abu)(Abu)L(Abu)LTVFL-PE
TS-5016-2	H-2D^{b} MoMSV Tetramer-(Abu)(Abu)L(Abu)LTVFL -APC
TS-5017-1	H-2D^{b} SIV gag Tetramer-AAVKNWMTQTL-PE
TS-5017-2	H-2D^{b} SIV gag Tetramer-AAVKNWMTQTL-APC

Foreign antigen

TS-5001-1C	H-2K^{b} OVA Tetramer-SIINFEKL-PE
TS-5001-2C	H-2K^{b} OVA Tetramer-SIINFEKL-APC
TS-M525-1	H-2K^{d} EGFP Tetramer-HYLSTQSAL-PE
TS-M503-1	H-2K^{d} Listeria LLO Tetramer-GYKDGNEYI-PE
TS-M515-1	H-2K^{d} malaria Tetramer-SYIPSAEKI-PE
TS-M517-1	H-2D^{d} BCG MPT51 Tetramer-GGPHAVYLL-PE
TS-M501-1	H-2K^{b} β-galactosidase Tetramer-DAPIYTNV-PE
TS-M501-2	H-2K^{b} β-galactosidase Tetramer-DAPIYTNV-APC
TS-M511-1	H-2L^{d} β-galactosidase Tetramer-TPHPARIGL-PE

Others

TS-M524-1	H-2D^{b} HY Uty Tetramer-WMHHNMDLI-PE
TS-M008-1	H-2K^{b} Negative Tetramer-SIYRYYGL-PE
TS-M704-1	I-A^{b} MOG$_{35-55}$ Tetramer-PE
TS-M707-1	I-A^{b} ESAT-6$_{1-20}$ Tetramer-PE
TS-MCD-1	Mouse CD1d Tetramer-PE

T-Select Peptides

TS-5001-P	H-2K^{b} OVA peptide
TS-5002-P	H-2D^{b} LCMV gp33 peptide
TS-M501-P	H-2K^{b} β-galactosidase peptide
TS-M502-P	H-2D^{b} Influenza NP 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 peptide
TS-M508-P	H-2D^{b} Influenza NP 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-M520-P	H-2K^{d} Influenza HA peptide
TS-M521-P	H-2L^{d} MuLV gp70 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-M527-P	H-2D^{b} Influenza NP peptide
TS-M528-P	H-2D^{b} Influenza PA 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 Tax38-46 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^{d} OVA 323-339 helper peptide
TS-M704-P	I-A^{b} MOG peptide
TS-M707-P	I-A^{b} ESAT-6 peptide
TS-M708-P	I-A^{k} HEL peptide

Kit

AM-1005	IMMUNOCYTO Cytotoxity Detection Kit

Others

D271-4	mouse CD8-FITC (KT15)
D271-A64	mouse CD8-Alexa Fluor® 647 (KT15)
732121	mouse CD16/32 (93)
732151	mouse CD45-APC (I3/2.3)
732152	mouse CD45-SPRD (I3/2.3)
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.

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