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 with 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 0.1% ProClin™.

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-Brilliant Violet™ 421 (SA-BV421)
  Excitation maximum 405 nm
  Emission maximum 421 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 (BV421 Tetramer) to pink (PE Tetramer), light blue (APC Tetramer) liquid.

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. 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 pipet 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-mouse CD8-FITC (clone KT15), MBL, PN K0227-4
- Anti-mouse CD8-Alexa Fluor™ 647 (clone KT15), MBL, PN K0227-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. 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. 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.
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 severalhours.

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.

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