

T-Select MHC Class I Human Tetramer

Allele and Peptide Specificity

The T-Select MHC Class I Human Tetramers recognize human CD8⁺ T cells which are specific for a particular peptide in combination with the HLA allele. The HLA molecule in this reagent has been modified to minimize CD8 mediated binding¹⁾.

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 HLA class I molecules each bound to the specific peptide^{2), 3)} 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/mononuclear cell preparations. 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.

High Specificity

The T cell surface CD8 enhances T cell antigen recognition by binding to HLA class I molecules. Therefore, MBL produced T-Select MHC class I human Tetramers with one point mutation at the HLA α 3 domain known to alter the interaction with CD8. These mutated Tetramers showed a greatly diminished nonspecific binding but retained specific binding. Alterations of CD8 binding by mutation of the MHC greatly improved the specificity of MHC-peptide multimers, thus providing efficient tools to sort specific human T cells for immunotherapy.

Reagents

500 μ L liquid - 10 μ L/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% NaN₃.

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.

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-CD8a (Human) mAb-FITC, MBL, PN K0226-4
- 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 μ L of T-Select MHC Tetramer to each 12 x 75 mm test tube.
3. Add 200 μ L 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 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 μ L of sample is required for each T-Select MHC Tetramer determination.
2. Add 10 μ L of Clear Back (human FcR blocking reagent, MBL, PN MTG-001) to each 12 x 75 mm test tube.
3. Add 50 μ L PBMC into each test tube (e.g. 1×10^6

cells per tube).

4. Incubate for 5 minutes at room temperature.
5. Add 10 μ L 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.
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. Suspend the pellet in 500 μ L of FCM buffer and analyze it immediately, or suspend it in 0.5% paraformaldehyde/PBS and store the sample in a dark room at 2-8°C. Be sure to analyze it within 24 hours.

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 performed, we recommend the use of heparin as an anti-coagulant.
- B. 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.
- 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 anti-CD8 antibody, clone Hit8a (MBL, PN K0226-4) or SFC121Thy2D3 (T8) because some anti-CD8 antibodies inhibit Tetramer-specific binding to TCR.
- 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) Bodinier M, Peyrat M-A, Tournay C, Davodeau F, Romagne F, Bonneville M, and Lang F, 2000. Efficient Detection and Immunomagnetic Sorting of Specific T Cells Using Multimers of MHC Class I and Peptide with Reduced CD8 Binding. *Nat. Med.*, 6:707-710.
- 2) 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.
- 3) McMichael AJ, and O'Callaghan CA. 1998. A New Look at T Cells. *J. Exp. Med.* 187:1367-1371.

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Please check our web site (<https://ruo.mbl.co.jp>) for up-to-date information on products and custom MHC Tetramers.