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Not for use in diagnostic procedures.**

MBL A JSR Life Sciences Company

T-Select MHC Tetramer

I-A^b Mtb Ag85B$_{240-254}$ Tetramer -FQDAYNAAGGHNAVF (20 tests)

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Background

T lymphocytes play a central role in the 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 accomplished using T-Select MHC Class II Tetramers which are composed of four MHC class II molecules each bound to a specific peptide1, 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, Tetramer-stained 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 in which frequency is low, it may be necessary to perform an in vitro cell expansion3. 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.

I-Ab Mtb Ag85B240-254 Tetramer comprises mouse MHC class II I-Ab and peptide fragment of diacylglycerol acyltransferase/mycolyltransferase Ag85B from Mycobacterium tuberculosis.

M. tuberculosis is a species of pathogenic bacteria in the family Mycobacteriaceae and the causative agent of tuberculosis. Antigen 85 B (Ag85B) is secreted at the early phase of M. tuberculosis infection, leading to the proliferation of Ag85B-specific CD4+ T cells. Ag85B240-254 peptide (also known as Peptide-25) is the major mediator of CD4+ T cell cytokine production and proliferation in response to Ag85B.

I-Ab Mtb Ag85B240-254 Tetramer can be used for staining CD4+ T cells that are specific for Mtb Ag85B240-254 peptide for cell enumeration by flow cytometry in particular gating condition (see Page 3).

Allele: I-A^b

Peptide Sequence: Mtb Ag85B$_{240-254}$
"FQDAYNAAGGHNAVF" is a part of Diacylglycerol acyltransferase/mycolyltransferase Ag85B from *Mycobacterium tuberculosis.*

Usage

This reagent is for use with standard flow cytometry methodologies.

Reagents

200 μL liquid - 10 μL/test
T-Select MHC Class II Mouse Tetramer - 20 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

TS-M719-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M719-2
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.

Stability

This reagent is stable until the expiration date shown on the label under the recommended storage conditions.

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.

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), or light blue (SA-APC).

Mouse I-A alleles

MHC class II	I-A^b	I-A^d	I-A^k	I-A^S	I-A^{g7}
Mouse strains	C57BL/- BXSB/Mp 129/-	BALB/c DBA/2 B10.D2	C3H/He	SJL/J B10.S	NOD

References about Mtb $Ag85B_{240-254}$

1) Kariyone A, *et al. Int. Immunol.* **15**: 1183-1194 (2003)

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 pipette 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-CD4 (Mouse) mAb-FITC (clone GK1.5) MBL PN D341-4
- 7-AAD Viability Dye Beckman Coulter, Inc., PN A07704
- Clear Back (Human FcR blocking reagent), MBL, PN MTG-001

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 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 or room temperature (15-25°C) protected from light.
7. Add any additional antibodies (e.g. anti-mouse CD4) and vortex gently.
8. Incubate for 30 minutes at 2-8°C protected from light. If red blood cell lysis is necessary, lyse red blood cells using commercially available reagents.
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.

Cell Expansion

Cell expansion, in the presence or absence of carboxyfluorescein succinimidyl ester (CFSE) to determine precursor frequency, is performed according to established protocols[4, 5]. Cells should be resuspended at a final concentration of 5 x 10^6 cells/mL after expansion and harvesting. A 200 μL sample is required for each test.

Technical Hints

A. 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.

B. A Tetramer that is constructed with the same allele of interest and an irrelevant peptide may be used as a negative control.

C. 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.

D. 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).

E. 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, *et al*. *Science* **274**: 94-96 (1996)
2. McMichael AJ and O 'Callaghan CA, *J Exp Med* **187**: 1367-1371 (1998)
3. Nepom GT, *et al*. *Arthritis Rheum* **46**: 5-12 (2002)
4. Lyons AB and Doherty KV, *Current Protocols in Cytometry* **2**: 9.11.1-9.11.9 (1998)
5. Novak EJ, *et al*. *J Clin Ivest* **104**: R63-R67 (1999)

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Example of Tetramer Staining

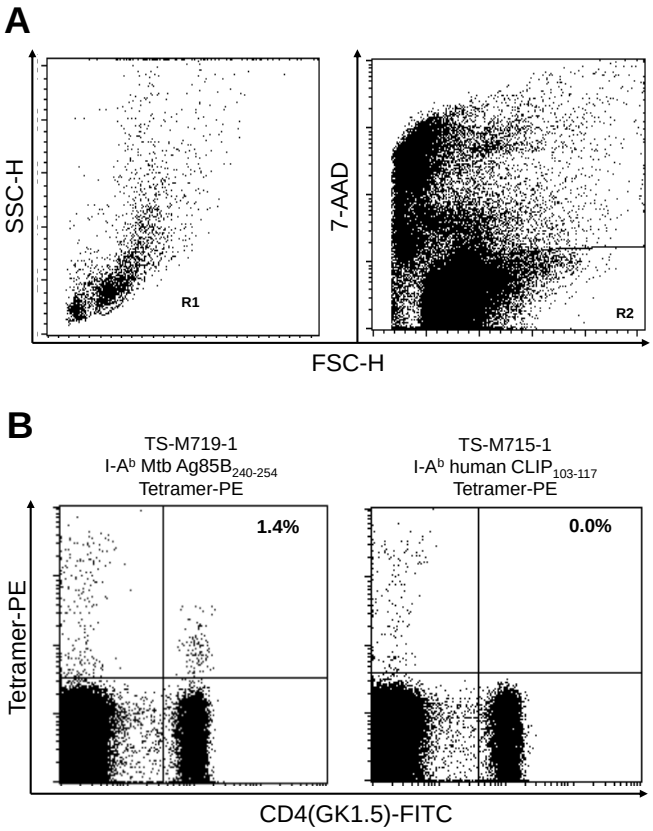
For example of Tetramer staining, C57BL/6 mice were immunized intraperitoneally with 100 nmol of the Mtb Ag85B$_{240-254}$ peptide (FQDAYNAAGGHNAVF, MBL, PN TS-M719-P) and 100 ng of Pertussis toxin (Wako) in complete Freund's adjuvant 2 times with 10 days intervals. Splenocytes were prepared from the immunized mice 11 days after the latest immunization and stained with the I-A^b Mtb Ag85B$_{240-254}$ Tetramer (Figure).

Procedure

1. Prepare peptide-immunized C57BL/6 splenocytes (2 x 10^6 cells). The splenocytes are hemolyzed with ACK lysis buffer and subsequently washed by FCM buffer (2% FCS/0.05% NaN_3/PBS) in each test tube.
2. Add 1 mL FCM buffer, and centrifuge at 400 x g for 5 minutes.
3. Aspirate the supernatant carefully. Add 10 μL of Clear Back (MBL, PN MTG-001) and 70 μL of FCM buffer. Incubate for 5 minutes at room temperature.
4. Add 10 μL of I-A^b Mtb Ag85B$_{240-254}$ Tetramer-PE (MBL, PN TS-M719-1) or I-A^b human CLIP$_{103-117}$ Tetramer-PE (MBL, PN TS-M715-1) as a negative control to each test tube and mix well. Incubate the cells for 60 minutes at 4°C.
5. Add 10 μL of Anti-CD4 (Mouse) mAb-FITC (clone GK1.5, MBL, PN D341-4) to each test tube and mix well. Incubate for 20 minutes at 4°C.
6. Add 1 mL FCM buffer, and centrifuge at 400 x g for 5 minutes.
7. Aspirate the supernatant carefully. Suspend the cells with 400 μL of FCM buffer.
8. Add 5 μL of 7-AAD (MBL, PN A07704) for the exclusion of nonviable cells in flow cytometric assays.
9. Analyze the prepared samples by flow cytometry.

Results

The lymphocyte population was defined by an FSC/SSC gate (R1), the viable cell population was defined by an FSC/7-AAD (R2) and the cell population defined by an expanded FSC/SSC gate was R3. Data were analyzed by double gating on the R1 and R2, or R3 and R2 (Figure A). The frequency of MHC Tetramer$^+$ and CD4$^+$ T cells is shown as a percentage of total CD4$^+$ T cells. Staining by I-A^b Mtb Ag85B$_{240-254}$ Tetramer was clearly observed in the R1/R2 double-gated splenocytes. (Figure B left) On the other hand, staining splenocytes with I-A^b human CLIP$_{103-117}$ Tetramer, the I-A^b Mtb Ag85B$_{240-254}$ Tetramer-positive CD4$^+$ T cells were not detected (Figure B right). I-A^b Mtb Ag85B$_{240-254}$ Tetramer clearly stains CD4$^+$ T cells specific for Mtb Ag85B$_{240-254}$ peptide in the more expanded FSC/SSC gate.



Figure; Example of Tetramer Staining
(A) Gating position. (B) Tetramer staining with I-A^b Mtb Ag85B$_{240-254}$ Tetramer or I-A^b human CLIP$_{103-117}$ Tetramer.