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

T-Select MHC class II mouse Tetramer

I-A^b Eα_{52-68} Tetramer-PE (20 tests)

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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 CD4$^+$ antigen-specific T cells requires cognate recognition of the T cell receptor (TCR) by a class II MHC/peptide complex. This can be done using T-Select MHC Class II Tetramers which are composed of four MHC class II 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, 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 where frequency is low, it may be necessary to perform an *in vitro* cell expansion[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.

This Tetramer reagent comprises mouse MHC class II I-A^b and an Eα_{52-68} peptide derived from the α chain of another mouse MHC class II: I-E. The I-Ab/Eα_{52-68} peptide complex is related to differentiation of CD4$^+$ T cells in the thymus, and is involved in diseases, such as systemic lupus erythematosus (SLE) and autoimmune thyroiditis[3),4),5)]. As the Eα_{52-68} peptide is conserved in humans, it is an important target for immunological research[5)]. Anti-MHC class II presenting the Eα_{52-68} peptide antibody (clone Y-Ae) is specific to the I-Ab/Eα_{52-68} complex, and is used in many studies. Moreover, transgenic mice expressing I-Ab/α_{52-68}-specific TCRs have been produced[1),2)]. Therefore, the I-A^b Eα_{52-68} Tetramer is expected to be an essential tool in future experiments.

A Tetramer, which is constructed with the same allele (I-A^b) of interest and an irrelevant peptide, may be used as a negative control Tetramer.

If it is difficult to use the negative control Tetramer, the use of a cell population that does not contain CD4$^+$ T cells expressing the desired TCR is recommended as a control.

MHC restriction: I-A^b

Peptide Sequence

Eα (52-68 aa, ASFEAQGALANIAVDKA)

References for Eα_{52-68}

1) Rudensky AY, *et al. Nature* **353**: 660-662 (1991)
2) Ignatowicz L, *et al. J. Immunol.* **154**: 3852-3862 (1995)
3) Grubin CE, *et al. Immunity* **7**: 197-208 (1997)
4) Martinez-Soria E, *et al. J. Immunol.* **181**: 3651-3657 (2008)
5) Brown NK, *et al. J. Immunol.* **180**: 7039-7046 (2008)

Mouse Strain I-A Haplotypes:

I-A allele	I-A^b	I-A^d	I-A^k	I-A^S
Mouse strains	C57BL/- BXSB/Mp 129/-	BALB/c DBA/2	C3H/He	SJL/J B10.S

Conjugates

TC-M706-1: Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 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, pink (PE Tetramer) liquid.

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 0.1% Proclin™ 150.

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 pipette 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
- mouse CD4-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 of 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/0.05% 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 µL 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. 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.

References for T-Select MHC Tetramer

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)

Related Products

Please check our website (https://www.mbl-chinawide.cn) for up-to-date information on products and custom MHC Tetramers.

Example of Tetramer Staining

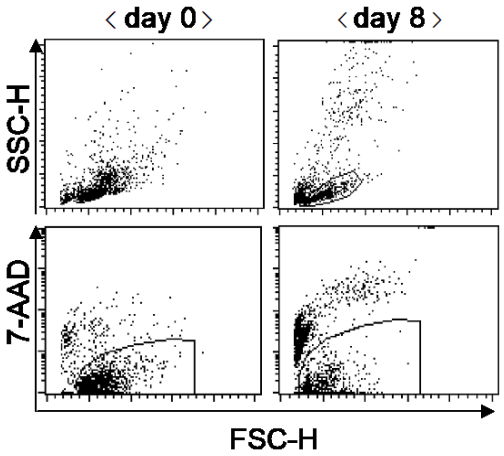
Mice were immunized intraperitoneally with 100 nmol of the I-A^b restricted E$\alpha_{52\text{-}68}$ peptide (ASFEAQGALANIAVDKA), 10 μg of cholera toxin in complete Freund's adjuvant 5 times. Splenocytes were prepared from the immunized mice 11 days after the latest immunization. Splenocytes were stained with MHC class II Tetramer on day 0. An aliquot of the splenocytes was cultured with the I-A^b restricted E$\alpha_{52\text{-}68}$ peptide for 8 days. Staining for MHC class II Tetramer was performed on day 8.

Procedure

1. Prepare immunized mice splenocytes or peptide stimulated cells (1 x 10^6 cells) hemolyzed with ACK lysis buffer, and wash in FCM buffer (2% FCS/0.05% NaN_3/PBS) in each test tube
2. Add 1 mL of FCM buffer, and centrifuge at 400 x g for 5 minutes.
3. Aspirate the supernatant carefully. Add 20 μL of Clear Back (MBL, PN MTG-001) and 20 μL of FCM buffer. Incubate for 5 minutes at room temperature.
4. Add 10 μL of I-A^b E$\alpha_{52\text{-}68}$ Tetramer-PE (MBL, PN TC-M706-1) or I-A^b OVA$_{323\text{-}339}$ Tetramer-PE as a negative control (MBL, PN TC-M710-1) to each test tube and mix well. Incubate the cells for 20 minutes at 4°C.
5. Add 10 μL of mouse CD4-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 in 400 μL of FCM buffer.
8. Add 5 μL of 7-AAD (Beckman Coulter, PN A07704) for the exclusion of nonviable cells in flow cytometric assays.
9. Analyze the prepared samples using flow cytometry.

Results

The lymphocyte population was defined by an FSC/SSC gate, and the viable cell population was defined by an FSC/7-AAD. Data were analyzed by double gating on the lymphocyte and viable cell population. The frequency of MHC Tetramer$^+$ and CD4$^+$ T cells was shown as a percentage of total CD4$^+$ T cells.



Limited staining was observed in freshly isolated splenocytes (day 0). However, the I-A^b E$\alpha_{52\text{-}68}$ Tetramer$^+$ CD4$^+$ T cells could be detected after *in vitro* stimulation (day 8). Tetramer$^+$ CD4$^+$ T cells were not detected in the negative control I-A^b OVA$_{323\text{-}339}$ Tetramer-PE.