

T-Select MHC Tetramer

# I-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer

## -QVYSLIRPNENPAHK (20 tests)

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

### 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<sup>+</sup> 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<sup>1, 2</sup> 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<sup>+</sup> 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<sup>3</sup>. 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-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer comprises mouse MHC class II I-A<sup>b</sup> and peptide fragment derived from nucleoprotein (NP) of influenza A virus. The nucleoprotein of influenza A virus is a structural protein that encapsidates the negative strand viral RNA and is essential for RNA transcription, replication and packaging. NP is one of the main determinants of species specificity. The Influenza NP<sub>311-325</sub> peptide fragment is derived from NP of influenza A and drives CD4<sup>+</sup> T cell response to influenza virus infection in mouse having I-A<sup>b</sup> allele.

I-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer can be used for staining CD4<sup>+</sup> T cells that is specific for influenza NP<sub>311-325</sub> peptide for cell enumeration by flow cytometry.

**Allele:** I-A<sup>b</sup>

**Peptide Sequence:** Influenza NP<sub>311-325</sub>  
"QVYSLIRPNENPAHK" derived from influenza A/PR8 virus nucleoprotein

### 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 0.1% Proclin™ 150.

### Conjugates

TC-M716-1  
Streptavidin-Phycoerythrin (SA-PE)  
Excites at 486-580 nm  
Emits at 586-590 nm

TC-M716-2  
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, pink (PE Tetramer) to light blue (APC Tetramer) liquid.

## Mouse I-A alleles

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

## References about Influenza NP<sub>311-325</sub>

- 1) Crowe S, *et al. Vaccine* **24**: 457-467 (2006)
- 2) Teijaro J, *et al. J. Virol* **84**: 9217-9226 (2010)
- 3) Ichihashi T, *et al. PLoS ONE* **8**: e60068 (2013)
- 4) Brincks EL, *et al. J Immunol* **190**: 3438-3446 (2013)
- 5) Martinez RJ, *et al. Nat Commun* **7**:13848 (2016)

## Statement of Warnings

- 1.
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
- 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  $2 \times 10^7$  cells/mL. 50  $\mu$ L of sample is

required for each T-Select MHC Tetramer determination.

2. Add 10  $\mu$ L of Clear Back (human FcR blocking reagent, MBL, PN MTG-001) to each 12 x 75 mm test tube.
3. Add 50  $\mu$ L cell suspension into each test tube (e.g.  $1 \times 10^6$  cells per tube).
4. Incubate for 5 minutes at room temperature.
5. Add 10  $\mu$ 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-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<sub>3</sub>/PBS).
10. Centrifuge tubes at 400 x g for 5 minutes.
11. Aspirate or decant the supernatant.
12. Resuspend the pellet in 500  $\mu$ L 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<sup>4,5</sup>. Cells should be resuspended at a final concentration of  $5 \times 10^6$  cells/mL after expansion and harvesting. A 200  $\mu$ L 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.

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

## **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 Invest* **104**: R63-R67 (1999)

## **Related Products**

### **T-Select Mouse class II Tetramers**

TC-M703-1 I-A<sup>d</sup> OVA<sub>323-339</sub> Tetramer-PE  
TC-M704-1 I-A<sup>b</sup> MOG<sub>35-55</sub> Tetramer-PE  
TC-M706-1 I-A<sup>b</sup> E<sub>α52-68</sub> Tetramer-PE  
TC-M707-1 I-A<sup>b</sup> ESAT-6<sub>1-20</sub> Tetramer-PE  
TC-M710-1 I-A<sup>b</sup> OVA<sub>323-339</sub> Tetramer-PE  
TC-M715-1 I-A<sup>b</sup> human CLIP<sub>103-117</sub> Tetramer-PE  
TC-M716-1 I-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer-PE  
TC-M720-1 I-A<sup>d</sup> human CLIP<sub>103-117</sub> Tetramer-PE  
TC-M722-1 I-A<sup>b</sup> mouse 2W1S Tetramer-PE

### **T-Select Human class II Tetramers**

TS-M801-1 HLA-DRB1\*01:01 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M802-1 HLA-DRB1\*01:01 HIV gag<sub>295-307</sub> Tetramer-PE  
TS-M803-1 HLA-DRB1\*01:01 EBV EBNA1<sub>515-527</sub> Tetramer-PE  
TS-M804-1 HLA-DRB1\*01:01 Influenza HA<sub>306-318</sub> Tetramer-PE  
TS-M805-1 HLA-DRB1\*04:05 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M806-1 HLA-DRB1\*04:05 Influenza HA<sub>306-318</sub> Tetramer-PE  
TS-M807-1 HLA-DRB1\*11:01 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M808-1 HLA-DRB1\*11:01 Influenza HA<sub>306-318</sub> Tetramer-PE  
TS-M809-1 HLA-DRB1\*04:01 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M810-1 HLA-DRB1\*04:01 Influenza HA<sub>306-318</sub> Tetramer-PE  
TS-M811-1 HLA-DRB1\*04:01 GAD65<sub>555-567</sub> Tetramer-PE  
TS-M812-1 HLA-DRB1\*11:01 TT p2<sub>829-844</sub> Tetramer-PE  
TS-M815-1 HLA-DRB1\*01:01 HTLV-1 Tax<sub>155-167</sub> Tetramer-PE  
TS-M816-1 HLA-DRB1\*15:01 human CLIP<sub>103-117</sub> Tetramer-PE  
TS-M817-1 HLA-DRB1\*15:02 human CLIP<sub>103-117</sub> Tetramer-PE

### **Others**

D341-4 mouse CD4-FITC (GK1.5)  
K0227-4 mouse CD8-FITC (KT15)  
K0227-5 mouse CD8-PE (KT15)  
K0227-A64 mouse CD8-Alexa Fluor® 647 (KT15)  
MTG-001 Clear Back (Human FcR blocking reagent)

## Example of Tetramer Staining

For example of Tetramer staining, C57BL/6 mice were immunized intraperitoneally with 100 nmol of the Influenza NP<sub>311-325</sub> peptide (QVYSLIRPNENPAHK) 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<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer at Day 0. Additionally, the splenocytes were stimulated with 10 µg/mL Influenza NP<sub>311-325</sub> peptide for 7 days in the presence of 100 U/mL recombinant human IL-2 and stained with the I-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer at Day 7 (Figure).

## Procedure

1. Prepare peptide-immunized C57BL/6 splenocytes or peptide-immunized C57BL/6 mouse splenocytes with 7 days *in vitro* peptide stimulation and cell expansion ( $2 \times 10^6$  cells). These splenocytes are hemolyzed with ACK lysis buffer and subsequently washed by FCM buffer (2% FCS/0.05% NaN<sub>3</sub>/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<sup>b</sup> Influenza A NP<sub>311-325</sub> Tetramer-PE (MBL, PN TC-M716-1) or I-A<sup>b</sup> MOG<sub>35-55</sub> Tetramer-PE (MBL, PN TC-M704-1) as negative control to each test tube and mix well. Incubate the cells for 60 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 with 400 µL of FCM buffer.
8. Add 5 µL of 7-AAD 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), and the viable cell population was defined by an FSC/7-AAD (R2). Data were analyzed by double gating on the lymphocyte and viable cell population (R1 and R2) (Figure A). The frequency of MHC Tetramer<sup>+</sup> and CD4<sup>+</sup> T cells was shown as a percentage of total CD4<sup>+</sup> T cells. Limited staining of Influenza NP<sub>311-325</sub>-specific CD4<sup>+</sup> T cells was observed in freshly isolated splenocytes (Day 0). After *in vitro* stimulation with the Influenza NP<sub>311-325</sub> peptide, the I-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer-positive CD4<sup>+</sup> T cells were clearly detected (Day 7) (Figure B). On the other hand, staining splenocytes with I-A<sup>b</sup> MOG<sub>35-55</sub> Tetramer, the I-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer-positive CD4<sup>+</sup> T cells were not detected (Figure B). I-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer is suitable for staining Influenza NP<sub>311-325</sub> specific CD4<sup>+</sup> T cells.

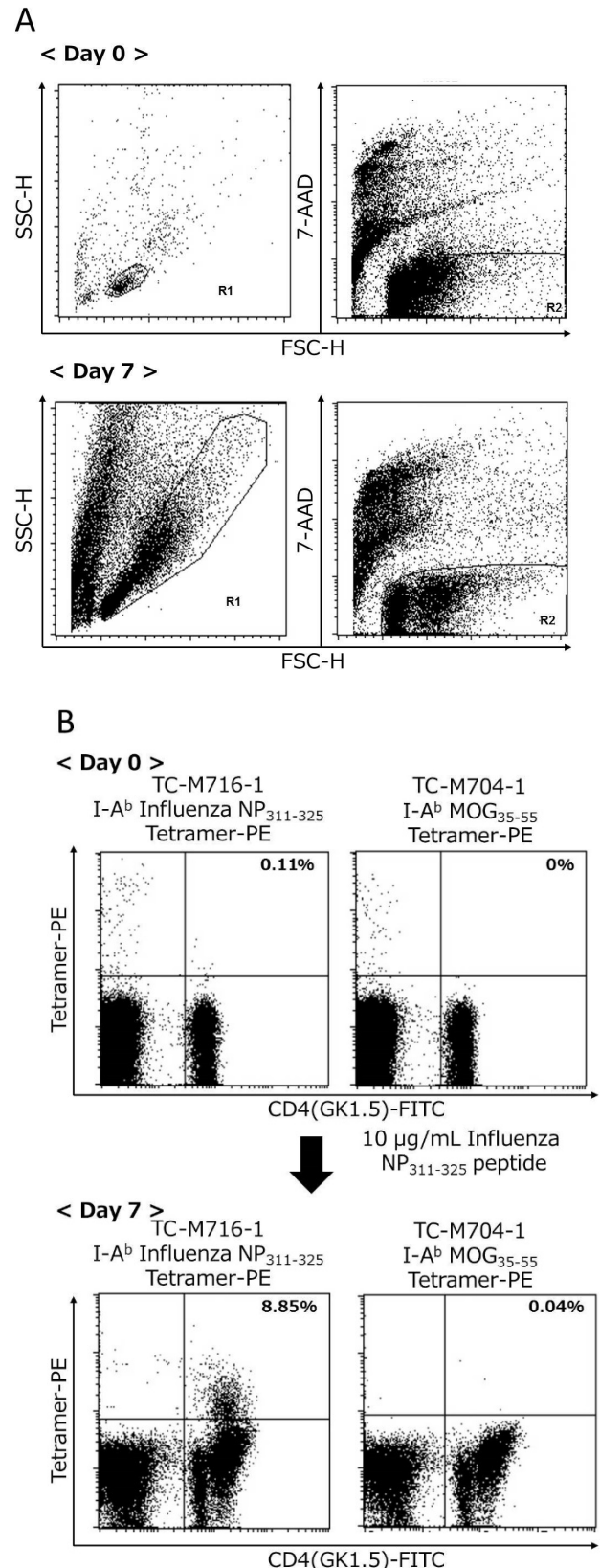


Figure Example of Tetramer Staining. (A) Gating position. (B) Tetramer staining with I-A<sup>b</sup> Influenza NP<sub>311-325</sub> Tetramer or I-A<sup>b</sup> MOG<sub>35-55</sub> Tetramer.