

T-Select MHC Tetramer

# H-2K<sup>d</sup> *Listeria* LLO Tetramer - GYKDGNEYI -PE (50 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 CD8<sup>+</sup> 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 T-Select MHC class I Tetramers which are composed of four MHC class I molecules each bound to the specific peptide 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 specific CD8<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. 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 murine class I MHC H-2K<sup>d</sup> and epitope peptide derived from *Listeria*, and it can detect an H-2K<sup>d</sup>-restricted *Listeria*-specific CD8<sup>+</sup> T cells. *Listeria monocytogenes* is a gram-positive bacterium. This epitope sequence is known to cause immunodeficiency through infection and growth in the cytoplasm. Live *Listeria*-infected mice have been useful for immunology research because CTL and Th1 are strongly induced as biodefence. Listeriolysin O (LLO)<sub>91-99</sub> is the most popular H-2K<sup>d</sup>-restricted peptide, and LLO-specific CTLs are immunodominant in *Listeria*-infected mice.

A Tetramer, which is constructed with the same allele (H-2K<sup>d</sup>) of interest and an irrelevant peptide, may be used as a negative control Tetramer.

## References for Products

- 1) Pamer EG, *et al. Nature* **353**: 852-855 (1991)
- 2) Busch DH, *et al. J. Exp. Med.* **188**: 61-70 (1998)
- 3) Huleatt JW, *et al. J. Immunol.* **166**: 4065-4073 (2001)
- 4) Mittrücker H-W, *et al. J. Immunol.* **167**: 5620-5627 (2001)

- 5) Yuan RR, *et al. Blood* **104**: 2397-2402 (2004)
- 6) Nishimura H, *et al. J. Immunol.* **175**: 4627-4634 (2005)
- 7) Li Z, *et al. Microbiology* **152**: 3091-3102 (2006)
- 8) Ozawa Y, *et al. Am. J. Respir. Cell Mol. Biol.* **41**: 440-448 (2009)
- 9) Hayashi T, *et al. J. Immunol.* **182**: 6360-6368 (2009)

## Mouse Strain H-2K Haplotypes:

H-2K allele	H-2K <sup>b</sup>	H-2K <sup>d</sup>	H-2K <sup>k</sup>
Mouse strains	C57BL/-, BXSB/Mp, 129/-	BALB/c, DBA/2, B10D2, NOD	CBA/ C3H/He, AKR/J

**MHC Restriction:** H-2K<sup>d</sup>

## Origin and Sequence of CTL Epitope

*Listeria* LLO (91-99 aa, GYKDGNEYI)

## Conjugates

TS-M503-1: Streptavidin-Phycoerythrin (SA-PE)  
Excites at 486-580 nm  
Emits at 586-590 nm

## 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 0.09% NaN<sub>3</sub>.

## 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 to pink (SA-PE).

## 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 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-mouse CD8-FITC (KT15), MBL, PN D271-4
- anti-mouse CD8-Alexa Fluor® 647 (KT15), MBL, PN D271-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 12x75 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  $2 \times 10^7$  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.  $1 \times 10^6$  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-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/0.05% NaN<sub>3</sub>/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. 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 that is constructed with the same allele of interest and an irrelevant peptide may be used as a negative control.
- D. We recommend the use of the CD8 antibody clone KT15 (MBL, PN D271-4), because some anti-CD8 antibodies inhibit Tetramer-specific binding to TCR.
- E. 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.
- F. 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).
- G. 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, *et al. J Exp Med* **187**: 1367-1371 (1998)
- 3) Bodinier M, *et al. Nat Med* **6**: 707-710 (2000)

### Related Products

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

### Experimental data

B10D2 mice were immunized with the H-2K<sup>d</sup> LLO peptide (GYKDGNEYI, MBL PN TS-M503-P) and I-A<sup>d</sup> Tetanus toxin p30 helper peptide (FNNFTVSFWLRVLPKVSASHLE, MBL PN TS-M702-P) in complete Freund's adjuvant subcutaneously two times. Splenocytes were prepared from the immunized mice after the latest immunization. Splenocytes were stained with MHC class I Tetramer on day 0. An aliquot of the splenocytes was cultured with the H-2K<sup>d</sup> LLO peptide (0.1 µg/mL or 1 µg/mL) for 7 days. Staining for MHC class I Tetramer was performed on day 7.

### Procedure:

1. Prepare immunized mice splenocytes or peptide stimulated cells ( $1 \times 10^6$  cells) hemolyzed with ACK lysis buffer, and wash in FCM buffer (2% FCS/0.05% NaN<sub>3</sub>/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 10 µL of Clear Back (MBL, PN MTG-001) and 30 µL of FCM buffer. Incubate for 5 minutes at room temperature.
4. Add 10 µL of H-2K<sup>d</sup> Listeria LLO Tetramer-GYKDGNEYI-PE (MBL, PN TS-M503-1) or H-2K<sup>d</sup> RSV Tetramer-SYIGSINNI-PE (MBL, PN TS-M506-1) as a negative control tetramer to each test tube and mix well. Incubate the cells for 20 minutes at 4°C.
5. Add 10 µL of mouse CD8-FITC (clone KT15, MBL, PN D271-4) to each test tube and mix well. Incubate for 20 minutes at 4°C.
6. Add 1 mL of 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 prepared samples using flow cytometry.

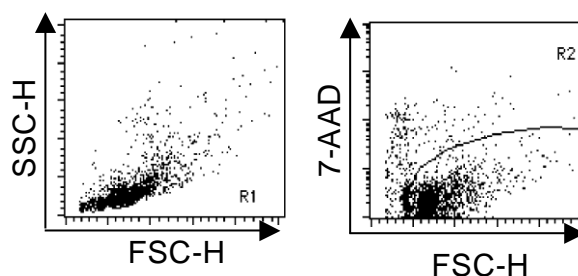
### Results:

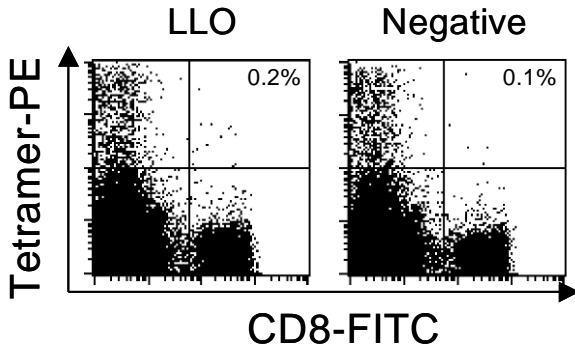
The lymphocyte population was defined by an FSC/SSC gate (R1), and the viable cell population was defined by an FSC/7-AAD (R2). Data were analyzed by double gating on the lymphocyte and viable cell population (R1 and R2).

The frequency of MHC Tetramer<sup>+</sup> and CD8<sup>+</sup> T cells is shown as a percentage of total CD8<sup>+</sup> T cells.

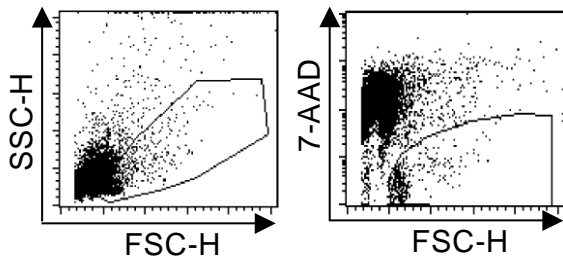
Limited staining was observed in freshly isolated splenocytes (day 0). However, the LLO Tetramer<sup>+</sup> CD8<sup>+</sup> T cells could be detected after *in vitro* stimulation (day 7). Tetramer<sup>+</sup> CD8<sup>+</sup> T cells were not detected in the negative control H-2K<sup>d</sup> RSV Tetramer-PE.

<day 0>

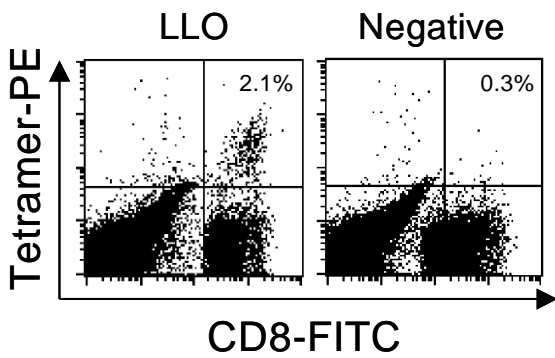




<day 7>



*in vitro* stimulation (peptide 0.1  $\mu\text{g/mL}$ )



*in vitro* stimulation (peptide 1  $\mu\text{g/mL}$ )

