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

T-Select MHC Tetramer

# I-A$^d$ human CLIP$_{103-117}$ Tetramer -PVSKMRMATPLLMQA (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.

I-A$^d$ human CLIP$_{103-117}$ Tetramer comprises mouse MHC class II I-A$^d$ and human class II-associated invariant chain peptide (CLIP), which is the part of the human invariant chain (Ii).

MHC class II is assembled in the ER of antigen presenting cell (APC) and pairs with the Ii. The Ii blocks peptide-binding groove of MHC class II to prevent it from binding cellular peptides or peptides from the endogenous pathway. The complex of MHC class II and Ii is transported to the late endosome, subsequently the Ii is degraded by resident protease, leaving only the CLIP. The remaining CLIP is exchanged with other epitope peptides with higher affinities.

I-A$^d$ human CLIP$_{103-117}$ Tetramer can be used as a negative control to the other Tetramers comprised of mouse MHC class II I-A$^d$ and different epitope peptides

## Allele: I-A$^d$

## Peptide Sequence: human CLIP$_{103-117}$

"PVSKMRMATPLLMQA" derived from human class II associated invariant chain (Ii)

## 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-M720-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M720-2
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 allele

| 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 human CLIP$_{103-117}$

1) Paul A, *et al. Nature* **345**: 615-618 (1990)
2) Victor S, *et al. Nature* **375**: 802-806 (1995)
3) Sebastian A, *et al*. *J Exp Med* **181**: 1729-1741 (1995)
4) Lisa K, *et al*. *J Cell* **82**: 155-165 (1995)
5) Felix B, *et al. PNAS* **98**: 12168-12173 (2001)
6) Cheryl LD, *et al. J Clin Invest* **112**: 831-842 (2003)
7) Gerald TN, *et al*. *J Immunol* **188**: 2477-2482 (2012)

## 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
- 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 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)

## Related Products

### T-Select Mouse class II Tetramers

TS-M703-1 I-$A^d$ $OVA_{323-339}$ Tetramer-PE
TS-M704-1 I-$A^b$ $MOG_{35-55}$ Tetramer-PE
TS-M705-1 I-$A^b$ $FMLV_{123-141}$ Tetramer-PE
TS-M706-1 I-$A^b$ E$\alpha_{52-68}$ Tetramer-PE
TS-M707-1 I-$A^b$ ESAT-$6_{1-20}$Tetramer-PE
TS-M710-1 I-$A^b$ $OVA_{323-339}$ Tetramer-PE
TS-M715-1 I-$A^b$ human $CLIP_{103-117}$ Tetramer-PE
TS-M716-1 I-$A^b$ Influenza $NP_{311-325}$ Tetramer-PE
TS-M720-1 I-$A^d$ human $CLIP_{103-117}$ Tetramer-PE
TS-M721-1 I-$A^b$ *L. monocytogenes* $LLO_{190-201}$ Tetramer-PE
TS-M722-1 I-$A^b$ mouse 2W1S Tetramer-PE
TS-M724-1 I-$A^b$ LCMV $GP_{126-140}$ Tetramer-PE

### T-Select Human class II Tetramers

TS-M801-1 HLA-DRB1*01:01 human $CLIP_{103-117}$ Tetramer-PE
TS-M802-1 HLA-DRB1*01:01 HIV $gag_{295-307}$ Tetramer-PE
TS-M803-1 HLA-DRB1*01:01 EBV $EBNA1_{515-527}$ Tetramer-PE
TS-M804-1 HLA-DRB1*01:01 Influenza $HA_{306-318}$ Tetramer-PE
TS-M805-1 HLA-DRB1*04:05 human $CLIP_{103-117}$ Tetramer-PE
TS-M806-1 HLA-DRB1*04:05 Influenza $HA_{306-318}$ Tetramer-PE
TS-M807-1 HLA-DRB1*11:01 human $CLIP_{103-117}$ Tetramer-PE
TS-M808-1 HLA-DRB1*11:01 Influenza $HA_{306-318}$ Tetramer-PE
TS-M809-1 HLA-DRB1*04:01 human $CLIP_{103-117}$ Tetramer-PE
TS-M810-1 HLA-DRB1*04:01 Influenza $HA_{306-318}$ Tetramer-PE
TS-M811-1 HLA-DRB1*04:01 $GAD65_{555-567}$ Tetramer-PE
TS-M812-1 HLA-DRB1*11:01 TT $p2_{829-844}$ Tetramer-PE
TS-M815-1 HLA-DRB1*01:01 HTLV-1 $Tax_{155-167}$ Tetramer-PE
TS-M816-1 HLA-DRB1*15:01 human $CLIP_{103-117}$ Tetramer-PE
TS-M817-1 HLA-DRB1*15:02 human $CLIP_{103-117}$ Tetramer-PE

### T-Select Peptides

TS-M701-P I-$A^b$ HBc helper peptide
TS-M702-P I-$A^d$ Tetanus toxin p30 helper peptide
TS-M703-P I-$A^b$/I-$A^d$ $OVA_{323-339}$ helper peptide
TS-M704-P I-$A^b$ $MOG_{35-55}$ peptide
TS-M707-P I-$A^b$ ESAT-$6_{1-20}$ peptide
TS-M708-P I-$A^k$ HEL peptide
TS-M716-P I-$A^b$ Influenza $NP_{311-325}$ peptide
TS-M721-P I-$A^b$ *L. monocytogenes* $LLO_{190-201}$ peptide
TS-M722-P I-$A^b$ mouse 2W1S peptide
TS-M724-P I-$A^b$ LCMV $GP_{126-140}$ peptide
TS-M801-P HLA-DRB1*01:01 human $CLIP_{103-117}$ peptide
TS-M802-P HLA-DRB1*01:01 HIV $gag_{295-307}$ peptide
TS-M803-P HLA-DRB1*01:01 EBV $EBNA1_{515-527}$ peptide
TS-M804-P HLA-DRB1*01:01 Influenza $HA_{306-318}$ peptide
TS-M811-P HLA-DRB1*04:01 $GAD65_{555-567}$ peptide
TS-M812-P HLA-DRB1*11:01 TT $p2_{829-844}$ peptide
TS-M815-P HLA-DRB1*01:01 HTLV-1 $Tax_{155-167}$ peptide

### Kit

AM-1005M IMMUNOCYTO Cytotoxicity Detection Kit
TB-7400-K1 QuickSwitch Quant H-2K$^b$ Tetramer Kit-PE
TB-7401-K1 QuickSwitch H-2K$^b$ Tetramer Kit-PE

### Others

D341-4 mouse CD4-FITC (GK1.5)
D271-4 mouse CD8-FITC (KT15)
D271-5 mouse CD8-PE (KT15)
D271-A64 mouse CD8-Alexa Fluor® 647 (KT15)
K0221-3 anti-mouse TCR DO11.10 (KJ1.26)
K0221-5 anti-mouse TCR DO11.10-PE (KJ1.26)
K0222-3 anti-mouse TCR 3DT-52.5 (KJ12.98)
A07704 7-AAD Viability Dye
MTG-001 Clear Back (Human FcR blocking reagent)

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

## Example of Tetramer Staining

For example of Tetramer staining, splenocytes were prepared from the B10.D2 mouse and stimulated with 1 μg/mL $OVA_{323-339}$ peptide for 6 days in the presence of 50 U/mL recombinant human IL-2. After the *in vitro* stimulation and cell expansion, the stimulated splenocytes were stained with the I-$A^d$ human $CLIP_{103-117}$ Tetramer or I-$A^d$ $OVA_{323–339}$ Tetramer (Figure).

## Procedure

1. Prepare peptide-immunized B10.D2 mouse splenocytes with *in vitro* peptide stimulation and cell expansion 6 days (2 x $10^6$ cells). These 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-$A^d$ human $CLIP_{103-117}$ Tetramer-PE (MBL, PN TS-M720-1) or I-$A^d$ $OVA_{323-339}$ Tetramer-PE (MBL, PN TS-M703-1) 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 (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), 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$^+$ and CD4$^+$ T cells is shown as a percentage of total CD4$^+$ T cells. The I-$A^d$ $OVA_{323-339}$ Tetramer clearly detected I-$A^d$ $OVA_{323-339}$ Tetramer-positive CD4$^+$ T cells from *in vitro* stimulated splenocytes with the $OVA_{323-339}$ peptide (Figure B). On the other hand, I-$A^d$ human $CLIP_{103-117}$ Tetramer did not stain I-$A^d$ $OVA_{323-339}$ Tetramer-positive CD4$^+$ T cells. (Figure B). I-$A^d$ human $CLIP_{103-117}$ Tetramer is suitable for the negative control Tetramer for other mouse I-$A^d$ Tetramers.

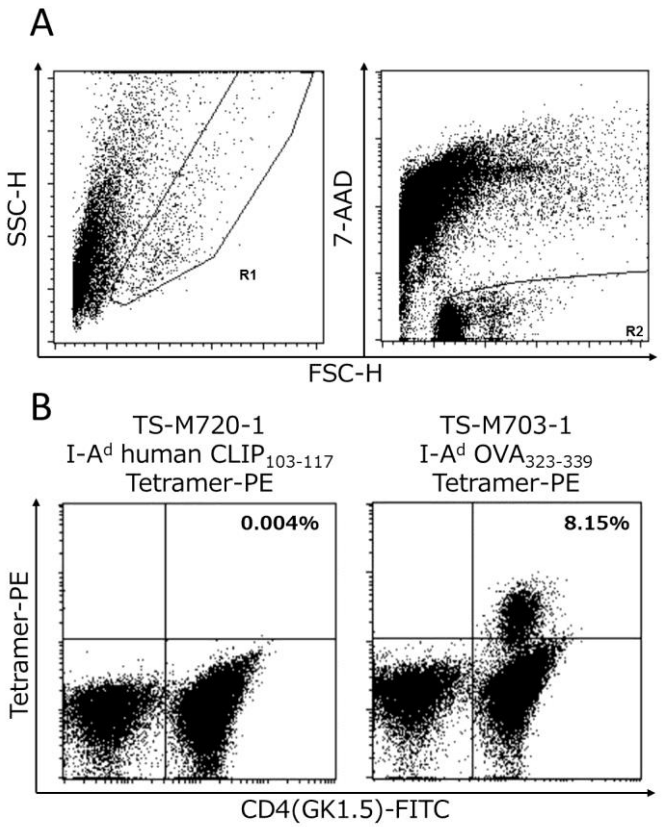


Figure Example of Tetramer Staining.
(A) Gating position. (B) Tetramer staining with I-$A^d$ human $CLIP_{103-117}$ Tetramer or I-$A^d$ $OVA_{323-339}$ Tetramer.