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

T-Select MHC Tetramer

I-A^b OVA$_{323-339}$ Tetramer -ISQAVHAAHAEINEAGR (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 murine class II MHC I-A^b and epitope peptide derived from ovalbumin (OVA), and it can detect an I-A^b-restricted OVA$_{323-339}$ -specific CD4$^+$ T cells. The CD4$^+$ T cell epitope, OVA$_{323-339}$, of the OVA model antigen has been a useful tool in immunology. The OT-II transgenic mouse strain carries a TCR transgene specific for the OVA$_{323-339}$ peptide, and it is useful for studying T cell immunology.

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. Alternatively, a cell population devoid of Tetramer-positive cells may be used as a negative control.

Storage Conditions

Store at 2 to 8°C. Do not freeze. Minimize exposure to light.

Precautions for Use

Please read this data sheet carefully with respect to the experimental conditions before using this product. A thorough understanding of the experimental data with regard to experimental conditions is required if you are planning to use OT-II TCR transgenic mice.

Allele: I-A^b

Peptide Sequence: OVA$_{323-339}$ peptide
"ISQAVHAAHAEINEAGR" derived from ovalbumin (OVA, 323-339 aa)

Usage

This reagent is for use with standard flow cytometry methodologies.

Reagents

200 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 0.1% Proclin™ 150.

Conjugates

TC-M710-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TC-M710-2
Streptavidin-Allophycocyanin (SA-APC)
Excites at 633-635 nm
Emits at 660-680 nm

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

References About $OVA_{323\text{-}339}$

1) Barnden MJ, *et al*. *Immunol Cell Biol* **76**: 34-40 (1998)
2) Moon JJ, *et al. Immunity* **27**: 203-213 (2007)
3) Landais E, *et al*. *J Immunol* **183**: 7949-7957 (2009)
4) Liao T-YA, *et al*. *PLoS ONE* **10**: e0145833 (2015)

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 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.

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.

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.

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 I OVA Tetramers

TS-5001-1C	H-2K^b OVA Tetramer-SIINFEKL-PE
TS-M541-1	H-2K^b OVA E1 Tetramer-EIINFEKL-PE
TS-M542-1	H-2K^b OVA G4 Tetramer-SIIGFEKL-PE
TS-M543-1	H-2K^b OVA Q4H7 Tetramer-SIIQFEHL-PE

T-Select Mouse Class II Tetramers

TC-M703-1	I-A^d OVA$_{323-339}$ Tetramer-PE
TC-M704-1	I-A^b MOG$_{35-55}$ Tetramer-PE
TC-M706-1	I-A^b Eα_{52-68} Tetramer-PE
TC-M707-1	I-A^b ESAT-6$_{1-20}$ Tetramer-PE
TC-M710-1	I-A^b OVA$_{323-339}$ Tetramer-PE
TC-M715-1	I-A^b human CLIP$_{103-117}$ Tetramer-PE
TC-M716-1	I-A^b Influenza A NP$_{311-325}$ Tetramer-PE
TC-M720-1	I-A^d human CLIP$_{103-117}$ Tetramer-PE
TC-M722-1	I-A^b mouse 2W1S 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-M813-1	HLA-DRB1*01:01 Fel d 1$_{49-66}$ Tetramer-PE
TS-M814-1	HLA-DRB1*04:01 Lol p 1$_{105-117}$ 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
TS-M818-1	HLA-DRB1*15:01 Bet v 1$_{142-156}$ Tetramer-PE

Others

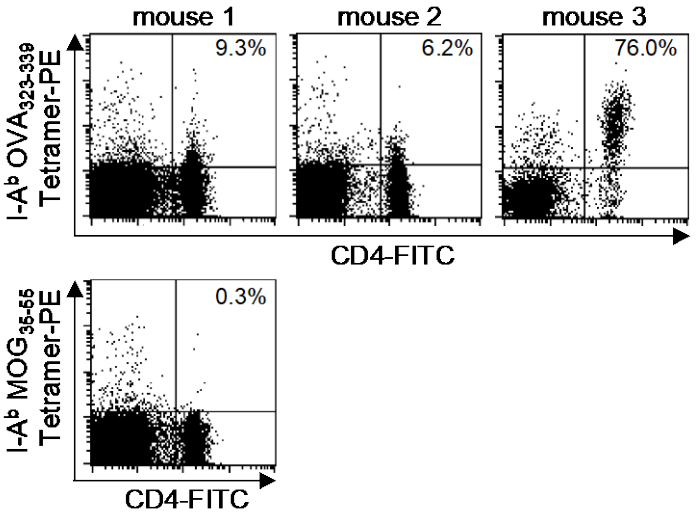
D341-4	mouse CD4-FITC (GK1.5)
K0227-4	mouse CD8-FITC (KT15)
K0227-5	mouse CD8-PE (KT15)
K0227-A64	mouse CD8-Alexa Fluor® 647 (KT15)
MTG-001	Clear Back (Human FcR blocking reagent)

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

Experimental data 1: *In vitro* peptide stimulation of OVA$_{323-339}$ specific TCR transgenic mice (OT-II).

The OT-II transgenic mouse strain has a TCR transgene specific for the OVA$_{323–339}$ peptide, and it is bred and maintained in the C57BL/6 genetic background. OT-II splenocytes were stained with the I-A^b OVA$_{323–339}$ Tetramer. Results showed that splenocytes from mouse 3 were recognized by the I-A^b OVA$_{323-339}$ Tetramer, whereas splenocytes from mouse 1 and 2 were not (results 1-1). This analysis revealed significant individual differences in staining with this Tetramer. We speculated that these differences were derived from low OVA-specific TCR expression. Therefore, splenocytes were specifically stimulated with OVA$_{323-339}$ peptide to induce TCR upregulation. Splenocytes from OT-II mice were stimulated with 0.1-10 μg/mL OVA$_{323-339}$ peptide for 6 days in the presence of 50 U/mL recombinant human IL-2. Staining with MHC class II-Tetramer was performed after stimulation (results 1-2). Data were analyzed by double gating on the lymphocyte and viable cell population (7-AAD negative). The frequency of MHC Tetramer$^+$ and CD4$^+$ cells is shown as a percentage of the total CD4$^+$ T cells.

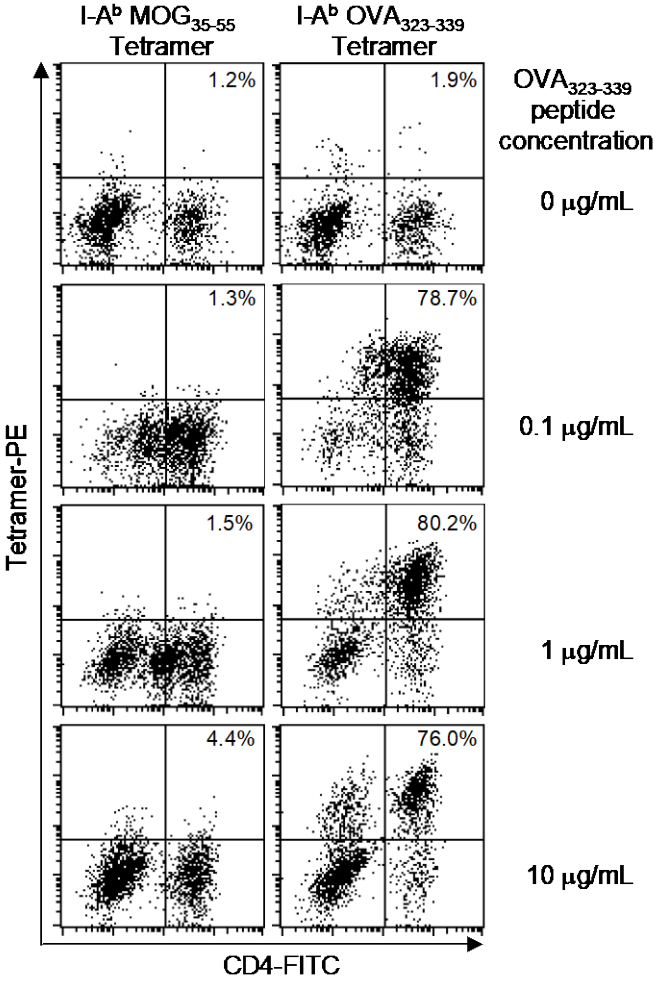
Results 1-1: Tetramer staining of freshly isolated OT-II splenocytes.



Splenocytes from mouse 3 were recognized by the I-A^b OVA$_{323-339}$ Tetramer, whereas splenocytes from mouse 1 and 2 were not.

Results 1-2: Tetramer staining of peptide -stimulated OT-II splenocytes (mouse 1) in culture.

Limited staining was observed in freshly isolated splenocytes. However, the I-A^b OVA$_{323-339}$ Tetramer-positive CD4$^+$ T cells could be detected after *in vitro* stimulation with the OVA$_{323-339}$ peptide. Tetramer-positive CD4$^+$ T cells were not detected in the negative control (I-A^b MOG$_{35-55}$ Tetramer).



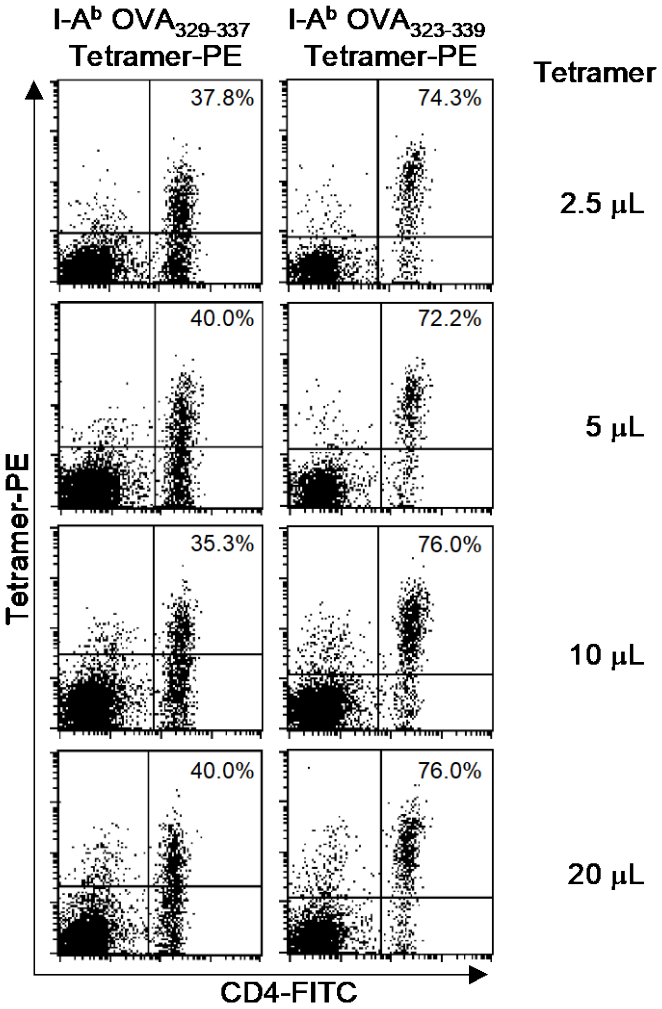
Experimental data 2: Comparison of populations observed by staining with two variants of I-A^b OVA Tetramer.

OT-II splenocyte preparations (mouse 3) were stained with two variants of I-A^b OVA Tetramer (I-A^b OVA$_{323-339}$ Tetramer-PE and I-A^b OVA$_{329-337}$ Tetramer-PE).

<Peptide sequence of I-A^b OVA Tetramer>
OVA$_{323-339}$: ISQAVHAAHAEINEAGR
OVA$_{329-337}$: AAHAEINEA

Results 2: Staining of freshly isolated OT-II splenocytes (mouse 3) with two Tetramer variants.

The frequency of MHC Tetramer$^+$ and CD4$^+$ T cells is shown as a percentage of total CD4$^+$ T cells.



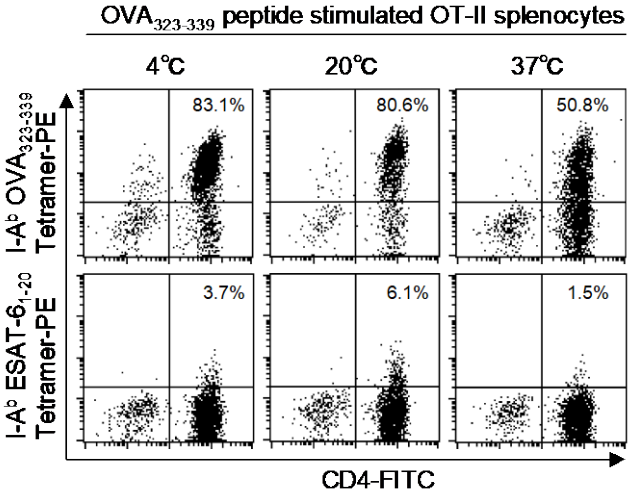
Although both I-A^b OVA Tetramer variants reacted with OT-II splenocytes (mouse 3), reactivity to the short epitope peptide I-A^b OVA$_{329-337}$ Tetramer was lower than that observed with the I-A^b OVA$_{323-339}$ Tetramer.

Experimental data 3: Determination of reaction temperature and Tetramer concentration.

OT-II splenocytes (mouse 1) were stimulated with 1 μg/mL of $OVA_{323-339}$ peptide for 6 days in the presence of 50 U/mL of recombinant human IL-2. The splenocytes were stained with MHC class II Tetramer after stimulation. Data were analyzed by double gating on the lymphocyte and viable cell population (7-AAD negative). The frequency of MHC Tetramer$^+$ and CD4$^+$ T cells is shown as a percentage of total CD4$^+$ T cells.

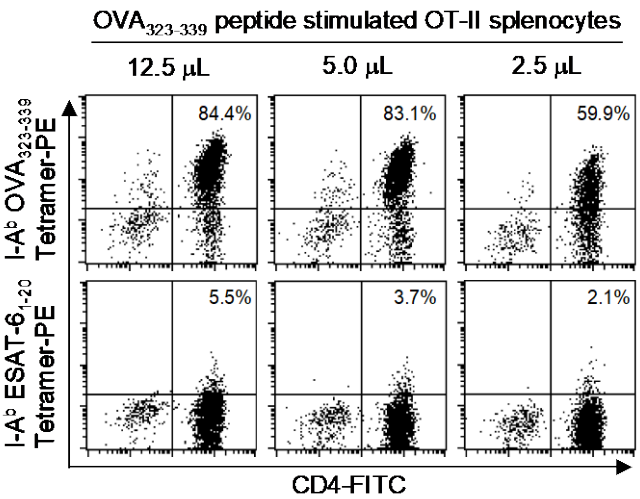
Results 3-1: Determination of the reaction temperature (Tetramer, 5 μL/tube)

The recommended I-A^b $OVA_{323-339}$ Tetramer assay conditions were determined as incubation for 60 min at 4°C, 20°C, or 37°C. Total reaction volume is 50 μL.



Results 3-2: Determination of the Tetramer concentration (reaction temperature, 4°C)

The optimal, recommended volume of I-A^b $OVA_{323-339}$ Tetramer was determined as approximately 5 μL/tube in a final reaction volume of 50 μL. Thus, we decided to recommend this Tetramer concentration.

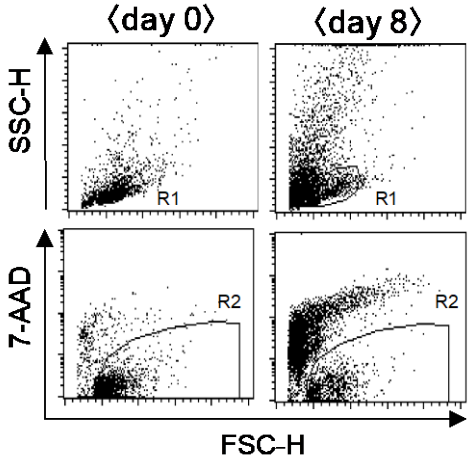


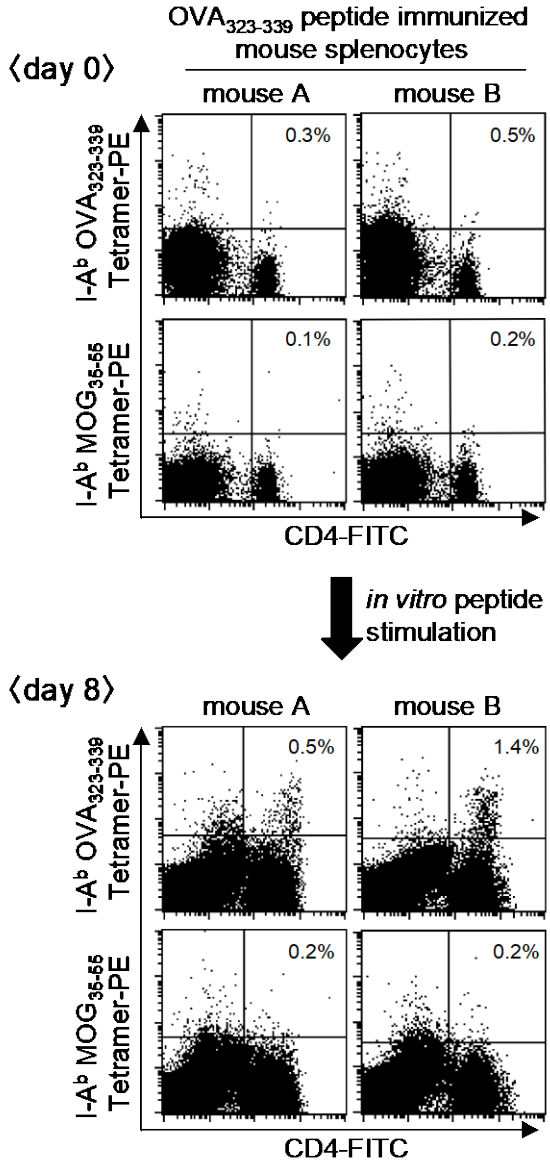
Experimental data 4: C57BL/6 mice immunized with $OVA_{323-339}$ peptide.

C57BL/6 mice were intraperitoneally immunized with 100 nmol $OVA_{323-339}$ peptide (ISQAVHAAHAEINEAGR,) and 10 μg cholera toxin in complete Freund's adjuvant. A second similar immunization was performed 10 days later. Splenocytes were prepared from the immunized mice 11 days after immunization. Splenocytes were stained with MHC class II Tetramer on day 0. An aliquot of the splenocytes was stimulated with 1 μg/mL $OVA_{323-339}$ peptide. After 48 hours, interleukin-2 (IL-2) was added to the cultures to a final concentration of 100 U/mL. Staining for MHC class II Tetramer was performed on day 8.

Results 4:

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$^+$ and CD4$^+$ T cells is shown as a percentage of total CD4$^+$ T cells.





Limited staining was observed in freshly isolated splenocytes. However, the I-A^b OVA$_{323-339}$ Tetramer-positive CD4$^+$ T cells could be detected after *in vitro* stimulation with the OVA$_{323-339}$ peptide. Tetramer-positive CD4$^+$ T cells were not detected in the negative control (I-A^b MOG$_{35-55}$ Tetramer).