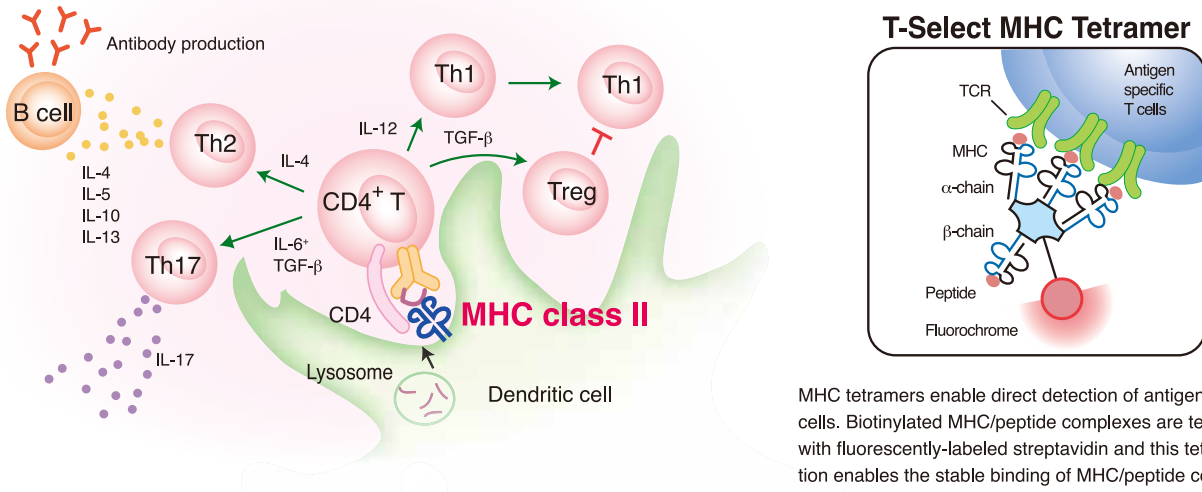


Mouse MHC class II Tetramer

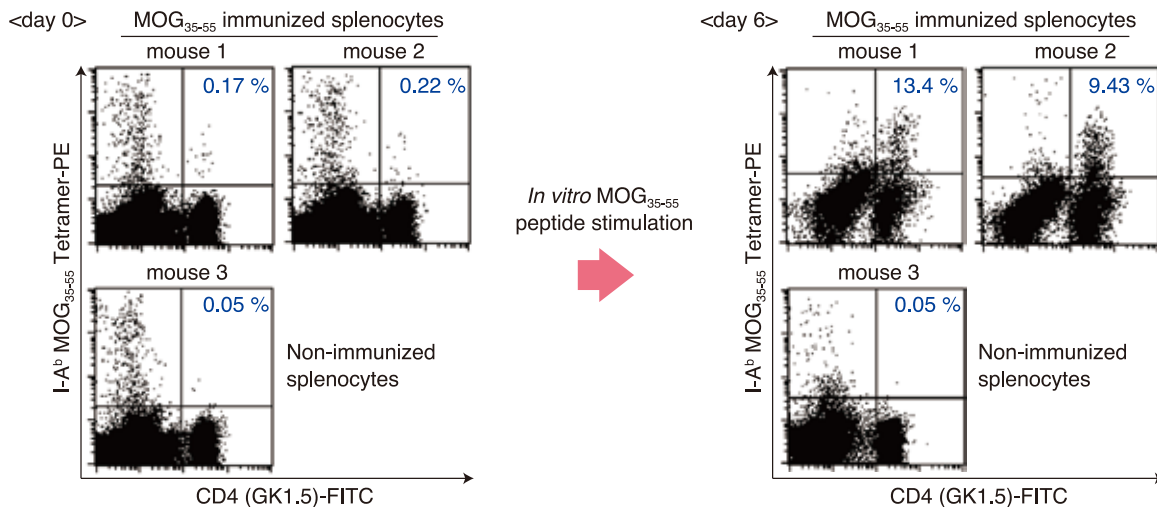
MHC Tetramers can be used for direct detection of antigen specific T cells.



MHC tetramers enable direct detection of antigen-specific T cells. Biotinylated MHC/peptide complexes are tetramerized with fluorescently-labeled streptavidin and this tetramerization enables the stable binding of MHC/peptide complexes to TCRs.

I-A^b MOG₃₅₋₅₅ Tetramer-PE

MOG₃₅₋₅₅ peptide is known as a useful tool to induce experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. This method is widely used as an experimental model of autoimmune diseases since the EAE symptoms appear soon after the animal is immunized with MOG₃₅₋₅₅ peptide and materials necessary for the experiment are relatively easy to obtain. It has been reported that regulatory T (Treg) cells and Th17 cells are involved in the onset of EAE. This suggests that immune balance of CD4⁺ T cells is important for the EAE pathogenesis.



C57BL/6 mice were immunized intraperitoneally twice with 100 nmol of I-A^b-restricted MOG₃₅₋₅₅ peptide (MEVGWYRSPFSRVVHLYRNGK, MBL code no. TS-M704-P) and 10 μg cholera toxin (MBL code no. RK-01-511) in complete Freund's adjuvant. 11 days later, splenocytes were prepared from the immunized mice. The isolated splenocytes were stained with I-A^b MOG₃₅₋₅₅ Tetramer-PE (MBL code no. TS-M704-1) on day 0. An aliquot of the splenocytes was stimulated with 10 μg/mL MOG₃₅₋₅₅ peptide for 6 days *in vitro*. The stimulated splenocytes were stained with MHC class II Tetramer on day 6. As a result, the I-A^b MOG₃₅₋₅₅ Tetramer-positive CD4⁺ T cells were detected after *in vitro* stimulation with the MOG₃₅₋₅₅ peptide (mouse 1 and 2). In case of negative control mouse splenocytes, the I-A^b MOG₃₅₋₅₅ Tetramer-positive CD4⁺ T cells were not detected (mouse 3). The number in the upper right of each dot plot indicates the frequency of MHC tetramer-positive CD4⁺ T cells in CD4⁺ T cells.

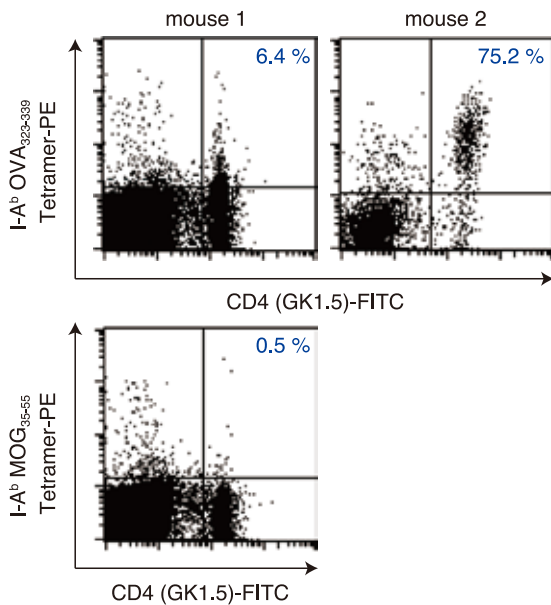
Code no.	Product	Size
TS-M704-1	T-Select I-A ^b MOG ₃₅₋₅₅ Tetramer-PE	20 tests
TS-M704-P	I-A ^b MOG ₃₅₋₅₅ Peptide	100 μL (10 mg/mL)

Code no.	Product	Clone	Isotype	Size
D341-4	Anti-CD4 (Mouse) mAb-FITC	GK1.5	Rat IgG2bk	1 mL (100 tests)

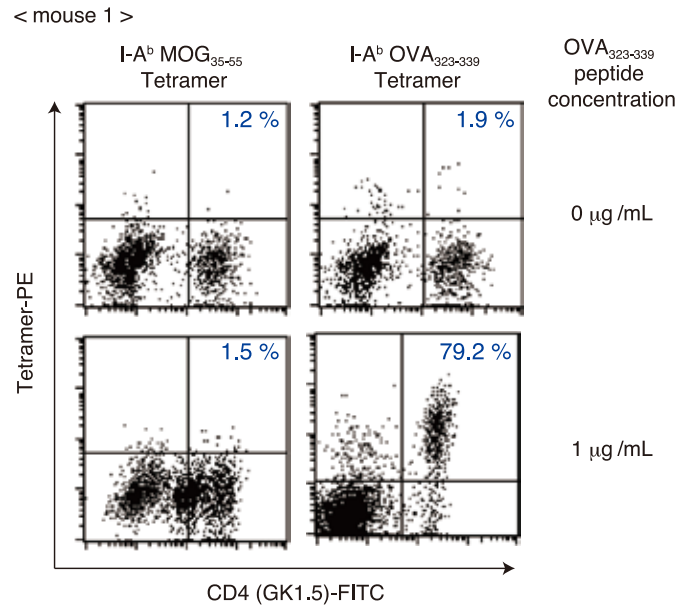
I-A^b OVA₃₂₃₋₃₃₉ Tetramer-PE

OVA has been used in many research fields as a model antigen that induce various immune responses. The OT-II transgenic mouse strain carries a TCR transgene specific for the OVA₃₂₃₋₃₃₉ peptide (ISQAVHAAHAEINEAGR), and this mouse strain contributes greatly to immunological studies as a tool for studying T-cell differentiation and immune responses. I-A^b OVA₃₂₃₋₃₃₉ Tetramer is expected to become an important tool for various experimental systems.

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



1-2 : Tetramer staining of peptide-stimulated OT-II splenocytes



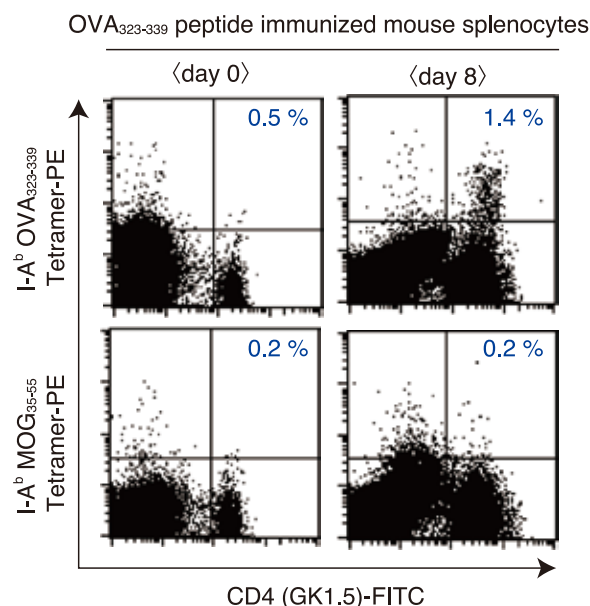
Freshly isolated OT-II splenocytes were stained with I-A^b OVA₃₂₃₋₃₃₉ Tetramer (1-1). Splenocytes from mouse 2 were recognized by the I-A^b OVA₃₂₃₋₃₃₉ Tetramer, whereas splenocytes from mouse 1 were not recognized.

We speculated that OVA-specific TCR expression of mouse 1 was low. Therefore, we analyzed whether stimulation with OVA₃₂₃₋₃₃₉ peptide upregulate the expression of TCR.

Splenocytes from mouse 1 were stimulated with 1 μg/mL OVA₃₂₃₋₃₃₉ peptide (ISQAVHAAHAEINEAGR, MBL code no. TS-M703-P) for 6 days. The samples were stained with MHC class II-Tetramers after stimulation (1-2).

As a result, the I-A^b OVA₃₂₃₋₃₃₉ Tetramer-positive CD4⁺ T cells were detected after *in vitro* stimulation with the OVA₃₂₃₋₃₃₉ peptide. Tetramer-positive CD4⁺ T cells were not detected in the negative control that was stained with I-A^b MOG₃₅₋₅₅ Tetramer.

Tetramer staining of OVA₃₂₃₋₃₃₉ peptide immunized mouse splenocytes



C57BL/6 mice were intraperitoneally immunized twice with 100 nmol OVA₃₂₃₋₃₃₉ peptide and 10 μg cholera toxin (MBL code no. RK-01-511) in complete Freund's adjuvant. 11 days after the immunization, splenocytes were prepared from the immunized mice. Splenocytes were stained with MHC class II Tetramers on day 0. An aliquot of the splenocytes was stimulated with 1 μg/mL OVA₃₂₃₋₃₃₉ peptide for 8 days *in vitro*. The stimulated splenocytes were stained with MHC class II Tetramers on day 8.

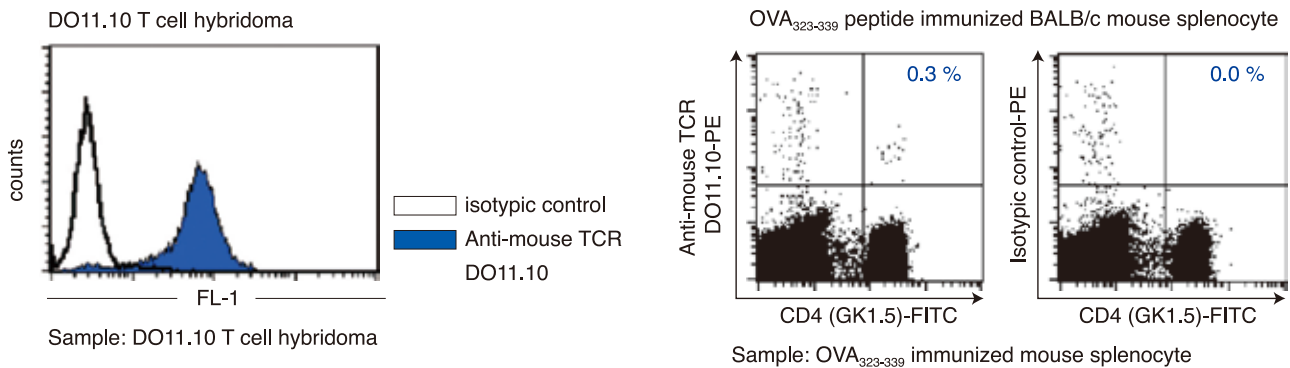
As a result, I-A^b OVA₃₂₃₋₃₃₉ Tetramer-positive CD4⁺ T cells were induced by *in vitro* stimulation with the OVA₃₂₃₋₃₃₉ peptide. Tetramer-positive CD4⁺ T cells were not detected in the negative control that was stained with I-A^b MOG₃₅₋₅₅ Tetramer.

Code no.	Product	Size
TS-M710-1	T-Select I-A ^b OVA ₃₂₃₋₃₃₉ Tetramer-PE	20 tests
TS-M703-P	I-A ^d OVA helper peptide	100 μL (10 mg/mL)

Antibody recognizing Mouse I-A^d / OVA₃₂₃₋₃₃₉ specific TCR

Anti-mouse TCR DO11.10 mAb (MBL code no. K0221-5, clone KJ1.26) is well known as an antibody recognizing I-A^d-restricted OVA₃₂₃₋₃₃₉-specific TCR. The OVA₃₂₃₋₃₃₉ peptide (ISQAVHAAHAEINEAGR, MBL code no. TS-M703-P) is used as a helper peptide to induce antigen-specific CTLs.

Flow cytometry with Anti-mouse TCR DO11.10 mAb



Code no.	Product	Clone	Isotype	Size
K0221-5	Anti-TCR DO11.10 (Mouse) mAb-PE	KJ1.26	Mouse IgG2ak	1 mL (50 tests)

I-A alleles of mouse strains:

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

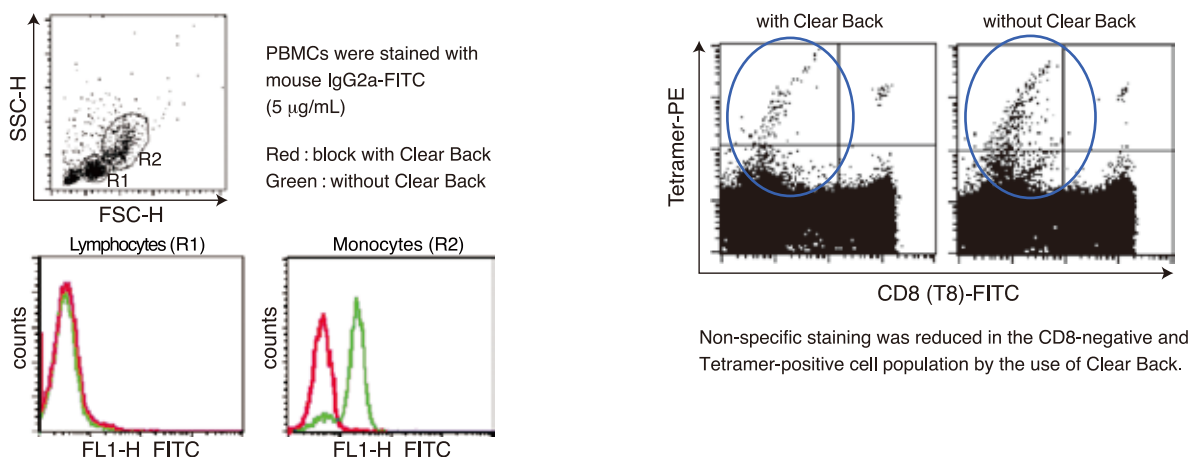
Clear Back (Human FcR blocking reagent) -Blocking reagent for tetramer staining-

Clear Back (MBL code no. MTG-001) is a reagent that blocks non-specific bindings of antibodies in experiments such as flow cytometry and immunofluorescence microscopy.

As indicated in the left figure below, Clear Back significantly reduces non-specific binding of antibodies to monocytes. Reduction of non-specific binding is especially important when detecting low frequency cell populations by tetramer reagents. Clear Back also inhibits the endocytosis by antigen-presenting cells such as macrophages and dendritic cells. This reagent is very easy to use and can be used for mouse samples as well as human samples.

The right figure below shows peripheral blood mononuclear cells (PBMCs) stained by HLA-A*24:02 EBV BRLF1 Tetramer with or without Clear Back. Clear Back blocks non-specific binding in tetramer-positive CD8-negative region.

Effect of Clear Back on PBMCs



Non-specific bindings were not detected on lymphocyte (R1). Clear Back blocked non-specific bindings of antibodies to monocytes (R2).

Non-specific staining was reduced in the CD8-negative and Tetramer-positive cell population by the use of Clear Back.

Code no.	Product	Size
MTG-001	Clear Back (Human Fc receptor blocking reagent)	1 mL (50 tests)x2

T-Select Mouse MHC class II Tetramers & related products

Mouse MHC class II Tetramer

Antigen	MHC	Sequence	Location (aa)	PE-labeled 20 tests	APC-labeled 20 tests	Peptide 100 µL (10 mg/mL)
MOG ₃₅₋₅₅	I-A ^b	MEVGWYRSPFSRVVHLYRNGK	35-55	TS-M704-1	Contact us	TS-M704-P
FMLV	I-A ^b	EPLTSLTPRCNTAWNRLKL	123-141	TS-M705-1		—
Eα ₅₂₋₆₈	I-A ^b	ASFEAQGALANIAVDKA	52-68	TS-M706-1		—
ESAT-6	I-A ^b	MTEQQWNFAGIEAAASAIQG	1-20	TS-M707-1		TS-M707-P
OVA	I-A ^b	ISQAVHAAHAEINEAGR	323-339	TS-M710-1		TS-M703-P

Mouse class II epitope peptide

Code no.	Sequence	Origin	Location (aa)	MHC	Size
TS-M701-P	TPPAYRPPNAPIL	HBc	128-140	I-A ^b	1 mg
TS-M702-P	FNNFTVSFWRVLPKVSASHLE	TT p30	947-967	I-A ^d	1 mg
TS-M703-P	ISQAVHAAHAEINEAGR	OVA	323-339	I-A ^b , I-A ^d	1 mg
TS-M704-P	MEVGWYRSPFSRVVHLYRNGK	MOG	35-55	I-A ^b	1 mg
TS-M707-P	MTEQQWNFAGIEAAASAIQG	ESAT-6	1-20	I-A ^b	1 mg
TS-M708-P	DGSTDYGILQINSRW	HEL	48-62	I-A ^k	1 mg

Mouse MHC class I OVA Tetramer

Antigen	MHC	Sequence	Location (aa)	PE-labeled 50 tests	APC-labeled 50 tests	Peptide 100 µL (10 mg/mL)
OVA	H-2K ^b	SIINFEKL	257-264	TS-5001-1C	TS-5001-2C	TS-5001-P
OVA E1	H-2K ^b	EIINFEKL	257-264	TS-M541-1	TS-M541-2	—
OVA G4	H-2K ^b	SIIGFEKL	257-264	TS-M542-1	TS-M542-2	—
OVA Q4H7	H-2K ^b	SIIQFEHL	257-264	TS-M543-1	TS-M543-2	—

Mouse CD1d Tetramer

Product	PE-labeled 50 tests	PE-labeled 10 tests	APC-labeled 50 tests
T-Select Mouse CD1d Tetramer	TS-MCD-1	TS-MCD-1S	TS-MCD-2

Antibody

Code no.	Product	Clone	Isotype	Size
D341-4	Anti-CD4 (Mouse) mAb-FITC	GK1.5	Rat IgG2bκ	1 mL (100 tests)
K0221-3	Anti-TCR DO11.10 (Mouse) mAb	KJ1.26	Mouse IgG2aκ	100 µg
K0221-5	Anti-TCR DO11.10 (Mouse) mAb-PE	KJ1.26	Mouse IgG2aκ	1 mL (50 tests)
K0222-3	Anti-TCR 3DT-52.5 (Mouse) mAb	KJ12.98	Mouse IgG2aκ	100 µg
M090-4	Rat IgG2b (isotype control)-FITC	3G8	Rat IgG2bκ	50 µg/1 mL
M076-3	Mouse IgG2a (isotype control)	6H3	Mouse IgG2aκ	100 µg/100 µL
M076-5	Mouse IgG2a (isotype control)-PE	6H3	Mouse IgG2aκ	10 µg/1 mL

Reagent

Code no.	Product	Size
A07704	7-AAD Viability Dye	150 tests
MTG-001	Clear Back (Human Fc receptor blocking reagent)	1 mL (50 tests)x2
IM-1400	OptiLyse B	250 tests
A11895	OptiLyse C	200 tests

Kit

Code no.	Product	Size
AM-1005	IMMUNOCYTO Cytotoxicity Detection Kit	50 tests