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MBL
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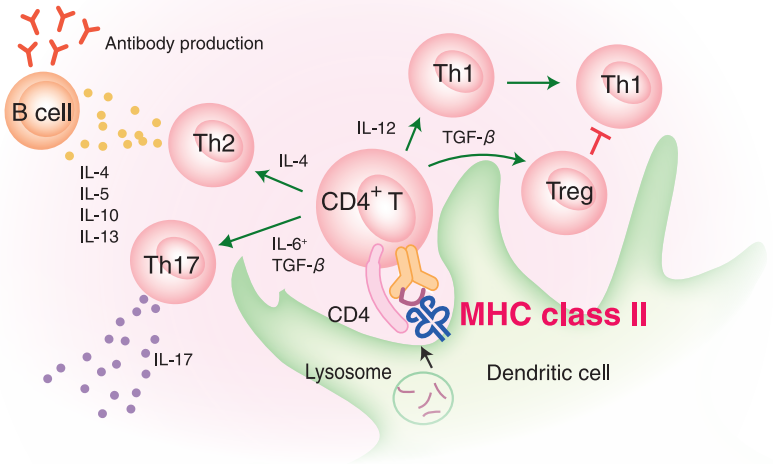
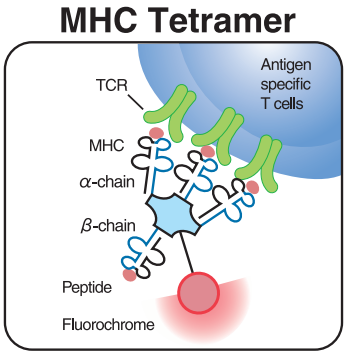
Mouse MHC class II Tetramer

MHC class II tetramers can be used for direct detection of antigen-specific CD4⁺ T cells.

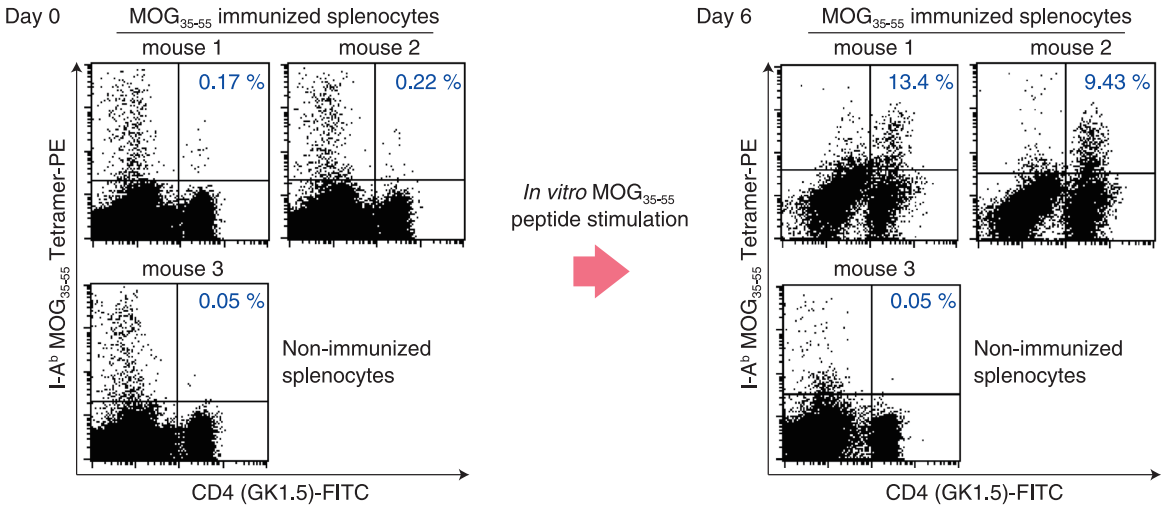




Biotinylated MHC/peptide complexes are tetramerized with fluorescently-labeled streptavidin, increasing the avidity of MHC/peptide complexes binding to TCR and allowing for direct detection of antigen-specific T cells.

I-A^b MOG$_{35-55}$ tetramer-PE

Experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis induced by immunization with MOG$_{35-55}$ peptide, is a widely used experimental model of autoimmune disease. It has been reported that regulatory T (Treg) cells and Th17 cells are involved in the onset of disease, suggesting that the immune balance of CD4⁺ T cells is important in EAE pathogenesis.



C57BL/6 mice were immunized intraperitoneally twice with 100 nmol of I-A^b-restricted MOG$_{35-55}$ peptide (MEVGWYRSPFSRVVHLYRNGK) and 10 μg cholera toxin in Freund's Complete Adjuvant. Eleven days later, splenocytes were isolated and stained with I-A^b MOG$_{35-55}$ tetramer-PE (MBL code no. TS-M704-1; Day 0). An aliquot of splenocytes was stimulated with 10 μg/mL MOG$_{35-55}$ peptide for 6 days *in vitro* and then stained with MHC class II tetramer (Day 6). *In vitro* stimulation with I-A^b MOG$_{35-55}$ resulted in increased tetramer-positive CD4⁺ T cells from immunized mice (mouse 1 and 2), but not from naïve mouse (mouse 3).

Code no.	Product	Size
TS-M704-1	T-Select I-A^b MOG$_{35-55}$ Tetramer-PE	20 tests

Code no.	Product	Clone	Isotype	Size
D341-4	Anti-CD4 (Mouse) mAb-FITC	GK1.5	Rat IgG2b κ	1 mL (100 tests)

I-A^b OVA$_{323-339}$ tetramer-PE

OVA is a model antigen commonly used to study immune responses in mice. OT-II transgenic mice express TCR specific for OVA$_{323-339}$ epitope (ISQAVHAAHAEINEAGR) in the context of I-A^b and serve as an important tool to study differentiation and activation of CD4 T cells. I-A^b OVA$_{323-339}$ tetramer can be used to monitor antigen-specific CD4 T cell responses in OT-II mice and in various experimental systems using OT-II cells for adoptive transfer.

Figure 1-1: I-A^b OVA$_{323-339}$ tetramer staining of freshly isolated OT-II splenocytes

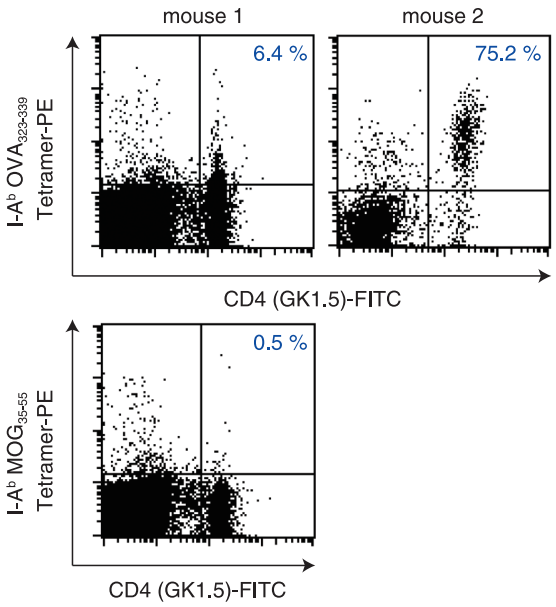
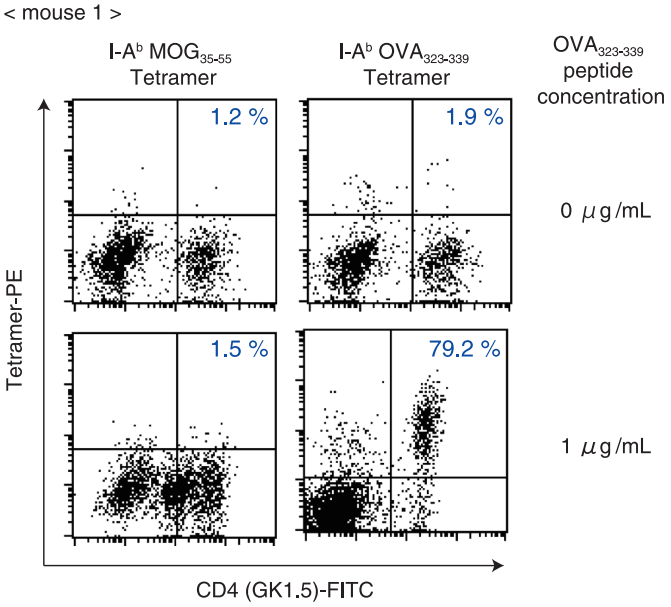
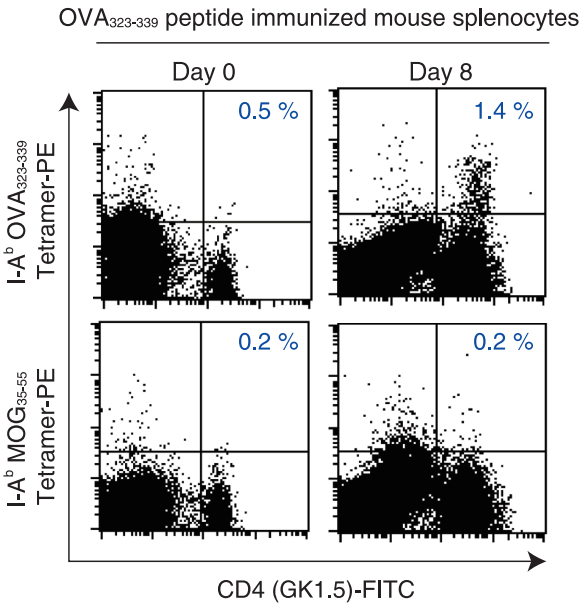


Figure 1-2: I-A^b OVA$_{323-339}$ tetramer staining of peptide -stimulated OT-II splenocytes (Day 6)



Reactivity of I-A^b OVA$_{323-339}$ tetramer reagent was assessed using freshly isolated spleen cells from OT-II mice. The majority of CD8 T cells were tetramer positive in mouse 2, but not in mouse 1 (Figure 1-1). *In vitro* stimulation with specific peptide significantly increased the number of tetramer positive cells (Figure 1-2).

Figure 2: I-A^b OVA$_{323-339}$ tetramer staining of splenocytes from peptide-immunized mice

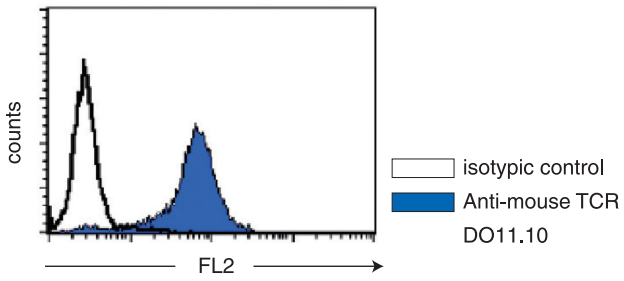


OVA-specific CD4 T cells can also be induced in wild-type mice. C57BL/6 mice were immunized twice IP with a mixture of 100 nmol of OVA$_{323-339}$ peptide and 10 μg of cholera toxin emulsified with adjuvant. After 11 days, spleen cells were harvested and cultured with a final concentration of 1 μg/mL OVA$_{323-339}$ peptide for 8 days. Cells were tested on days 0 and 8 of culture for OVA tetramer reactivity. In mice immunized with OVA$_{323-339}$ peptide, induction of specific T cells by *in vitro* peptide stimulation was confirmed using I-A^b OVA$_{323-339}$ tetramer. I-A^b MOG$_{35-55}$ tetramer, used as a negative control, showed no specific staining.

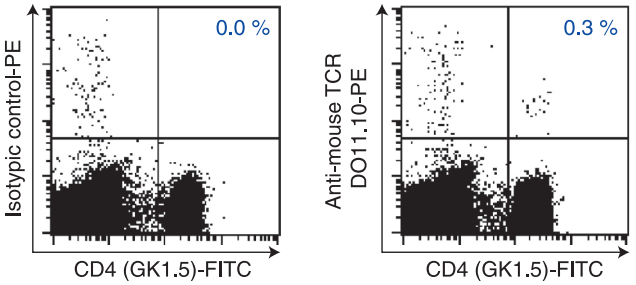
Antibody recognizing Mouse I-A^d / OVA$_{323-339}$ specific TCR

Anti-Mouse TCR DO11.10 (clone KJ1.26) is an antibody that binds to the TCR specific for OVA$_{323-339}$ presented in the context of I-A^d. This antibody can be used to identify OVA$_{323-339}$-specific CD4 T cells derived from I-A^d mice. OVA$_{323-339}$ peptide (ISQAVHAAHAEINEAGR) is often used as a helper peptide for induction of antigen-specific CTL in I-A^d-expressing mice.

Flow cytometry with Anti-mouse TCR DO11.10 mAb



Sample: DO11.10 T cell hybridoma



Sample: OVA$_{323-339}$ peptide immunized BALB/c mouse splenocytes

I-A alleles of mouse strains:

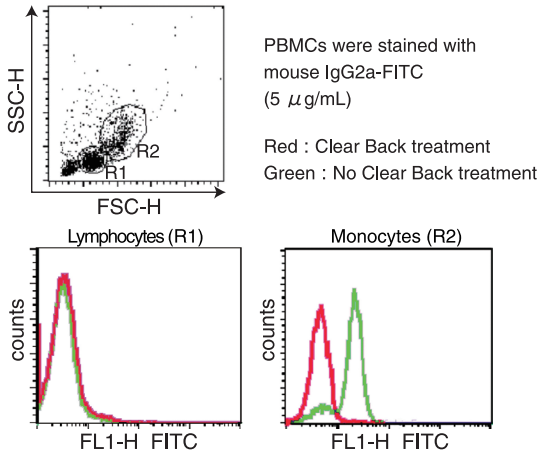
I-A allele	I-A^b	I-A^d	I-A^k	I-A^s
Mouse strains	C57BL/-, BXSB/Mp, 129/-	BALB/c, DBA/2	C3H/He	SJL/J, B10.S

Clear Back blocking reagent for tetramer staining

When a very rare cell population is detected by a tetramer reagent, it is important to reduce non-specific and Fc-specific staining. Clear Back (MBL code no. MTG-001) is a reagent that blocks non-specific binding to both human and murine cells in flow cytometry and fluorescence microscopy experiments. As shown in Figure 1, Clear Back reduced Fc-specific staining of monocytes by a mouse IgG2a. Clear Back also reduced background staining of peripheral blood mononuclear cells (PBMC) with HLA-A*24:02 EBV BRLF1 tetramer in the tetramer-positive CD8-negative region (Figure 2).

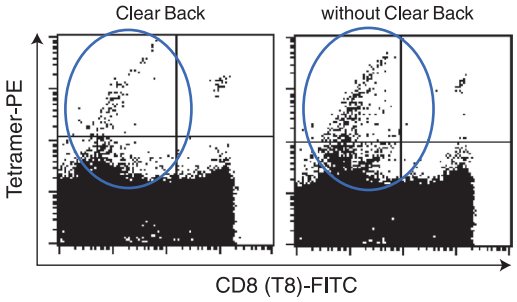
Effect of Clear Back on PBMCs

Figure 1



Non-specific binding of antibody was not detected on lymphocyte (R1). Clear Back treatment reduced unwanted Fc-specific staining of monocyte (R2) by a mouse IgG2a.

Figure 2



Clear Back treatment reduced non-specific binding of a tetramer reagent to tetramer-positive CD8-negative cell population.

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
MOG_{35-55}	I-A^b	MEVGWYRSPFSRVVHLYRNGK	35-55	TS-M704-1	Contact us
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	
OVA	I-A^b	ISQAVHAAHAEINEAGR	323-339	TS-M710-1	
OVA	I-A^d	ISQAVHAAHAEINEAGR	323-339	TS-M703-1	TS-M703-2

Mouse MHC class I OVA Tetramer

Antigen	MHC	Sequence	Location (aa)	PE-labeled 50 tests	APC-labeled 50 tests
OVA	H-$2K^b$	SIINFEKL	257-264	TS-5001-1C	TS-5001-2C
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	APC-labeled 50 tests
T-Select Mouse CD1d Tetramer	TS-MCD-1	TS-MCD-2

Antibody

Code no.	Product	Clone	Isotype	Size
D341-4	Anti-CD4 (Mouse) mAb-FITC	GK1.5	Rat IgG2b κ	1 mL (100 tests)
M090-4	Rat IgG2b (isotype control)-FITC	3G8	Rat IgG2b κ	50 μg/1 mL
M076-3	Mouse IgG2a (isotype control)	6H3	Mouse IgG2a κ	100 μg/100 μL
M076-5	Mouse IgG2a (isotype control)-PE	6H3	Mouse IgG2a κ	10 μg/1 mL

Reagent

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

For research use only. Not for use in diagnostic or therapeutic procedures.
The information is as of January 2024. Please contact us for the latest information. Please read the data sheets carefully before use.

2024.01 155188-24011000

Produced by

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