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Not for use in diagnostic procedures.**

MBL
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T-Select MHC Tetramer

I-A^d OVA$_{323-339}$ Tetramer (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, this 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^d and epitope peptide derived from ovalbumin (OVA), and it can detect an I-A^d-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 DO11.10 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^d) 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.

Allele: I-A^d

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

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.

Precautions for Use

These I-A^d OVA$_{323-339}$ Tetramers do not bind to DO11.10 TCR which is specific for OVA$_{323-339}$ presented by I-A^d. On the other hand, Anti-TCR DO11.10 (Mouse) mAb (MBL, PN K0221-3) and Anti-TCR DO11.10 (Mouse) mAb-PE (MBL, PN K0221-3) can detect DO11.10 TCR.

Our experimental data indicates that these I-A^d OVA$_{323-339}$ Tetramers and anti-mouse TCR DO11.10 antibody can detect different T cell populations.*

Please read this data sheet carefully with respect to the experimental conditions before using these products. *Please refer to **Experimental data 3**.

Conjugates

TS-M703-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M703-2
Streptavidin-Allophycocyanin (SA-APC)
Excites at 633-635 nm
Emits at 660-680 nm

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 0.09% NaN_3.

Usage

This reagent is for use with standard flow cytometry methodologies.

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.

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).

References About $OVA_{323-339}$

1) Shimonkevitz R, *et al. J Immunol* **133**: 2067-2074 (1984)
2) Barnden MJ, *et al*. *Immunol Cell Biol* **76**: 34-40 (1998)
3) Robertson MJ, *et al. J Immunol* **164**: 4706-4712 (2000)
4) Moon JJ, *et al. Immunity* **27**: 203-213 (2007)
5) Landais E, *et al*. *J Immunol* **183**: 7949-7957 (2009)

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

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
- MHC Tetramer Lyse Reagent, MBLI, PN T08002
- MHC Tetramer Fixative Reagent, MBLI, PN T08003
- Anti-mouse CD4-FITC (clone GK1.5), MBL, PN D341-4
- Clear Back (human FcR blocking reagent) MBL, PN MTG-001

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.

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) 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 (20°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, proceed to step 1) - 5).
   If red blood cell lysis is not necessary, continue to step 9 below.
   1) Lyse red blood cells using 1 mL of Lyse Reagent supplemented with 25 µL Fixative Reagent per tube.
   2) Vortex for 5 seconds immediately after the addition of the Lyse/Fixative solution per tube.
   3) Incubate for a minimum of 10 minutes at room temperature protected from light.
   4) Centrifuge tubes at 150 x g for 5 minutes.
   5) Aspirate or decant the supernatant.
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.

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. 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

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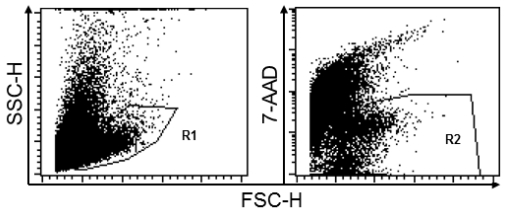
Experimental data 1

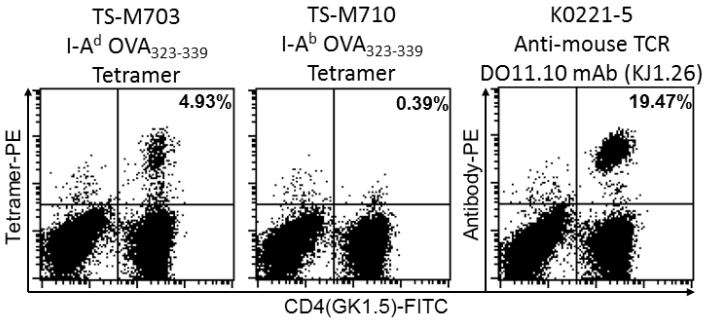
B10.D2 mice immunized with $OVA_{323-339}$ peptide.

B10.D2 mice were intraperitoneally immunized with 100 nmol $OVA_{323-339}$ peptide (ISQAVHAAHAEINEAGR, MBL, PN TS-M703-P) and 10 μg cholera toxin in complete Freund's adjuvant. A second similar immunization was performed 15 days later. Splenocytes were prepared from the immunized mice 14 days after immunization. Aliquots of the splenocytes were stimulated with 1 μg/mL $OVA_{323-339}$ peptide for 13 days. On day 2, recombinant human IL-2 was added (final concentration: 50 U/mL). Staining for MHC class II Tetramer was performed on day 13.

Protocols

1. Aliquots of the splenocytes stimulated OVA peptide for 13 days 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) 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 following reagents each and vortex gently.
   - I-A^d $OVA_{323-339}$ Tetramer-PE
   - I-A^b $OVA_{323-339}$ Tetramer-PE (negative control)
   - Anti-TCR DO11.10 antibody-PE
6. Incubate for 60 minutes at 2-8°C protected from light.
7. Add 10 μL anti-mouse CD4-FITC (GK1.5) antibody and vortex gently.
8. Incubate for 20 minutes at 2-8°C protected from light.
9. Add 1 mL of 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 FCM buffer (2% FCS/0.09% NaN_3/PBS).





Results 1

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.
The I-A^d $OVA_{323-339}$ 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 $OVA_{323-339}$ Tetramer).
The anti-TCR DO11.10 antibody-positive CD4$^+$ T cells also could be detected.

Experimental data 2

Comparison of staining ability between I-A^d OVA$_{323-339}$ Tetramer and I-A^d OVA$_{329-337}$ Tetramer.

B10.D2 mice were intraperitoneally immunized with 100 nmol OVA$_{323-339}$ peptide (ISQAVHAAHAEINEAGR, MBL, PN TS-M703-P) and 10 μg cholera toxin in complete Freund's adjuvant. A second similar immunization was performed 31 days later. Splenocytes were prepared from the immunized mice 14 days after immunization. Aliquots of the splenocytes were stimulated with 1 μg/mL OVA$_{323-339}$ peptide for 14 days. On day 3, recombinant human IL-2 was added (final concentration: 50 U/mL). Staining for MHC class II Tetramer was performed on day 14.

B10.D2 splenocytes preparations were stained with two variants of I-A^d OVA Tetramer (I-A^d OVA$_{323-339}$ Tetramer-PE and I-A^d OVA$_{329-337}$ Tetramer-PE).

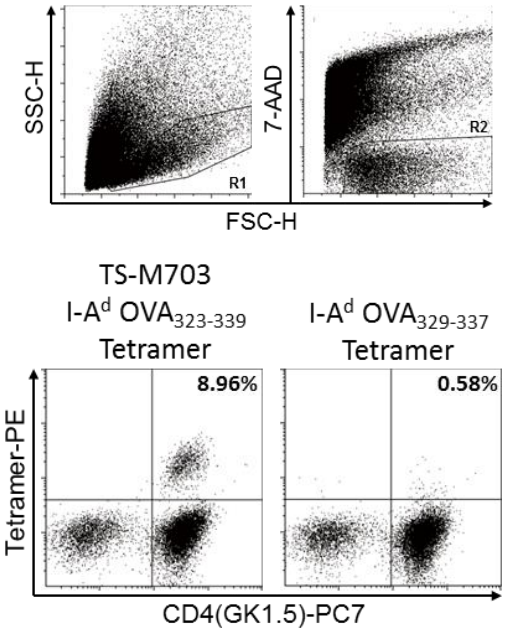
<Peptide sequence of I-A^d OVA Tetramer>
OVA$_{323-339}$: ISQAVHAAHAEINEAGR
OVA$_{329-337}$: AAHAEINEA

Protocols

1. Aliquots of the splenocytes stimulated OVA peptide for 14 days 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) 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 following reagents each and vortex gently.
   - I-A^d OVA$_{323-339}$ Tetramer-PE
   - I-A^d OVA$_{329-337}$ Tetramer-PE
6. Incubate for 60 minutes at 2-8°C protected from light.
7. Add 10 μL anti-mouse CD4 (GK1.5)-PE/Cy7 (PC7) antibody and vortex gently.
8. Incubate for 20 minutes at 2-8°C protected from light.
9. Add 1 mL of 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 FCM buffer (2% FCS/0.09% NaN_3/PBS).

Results 2

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.



Reactivity to the short epitope peptide I-A^d OVA$_{329-337}$ Tetramer was lower than that observed with the I-A^d OVA$_{323-339}$ Tetramer.

Experimental data 3

Comparison of $CD4^+$ T cell populations observed by staining with I-A^d $OVA_{323-339}$ Tetramer and anti-TCR DO11.10.

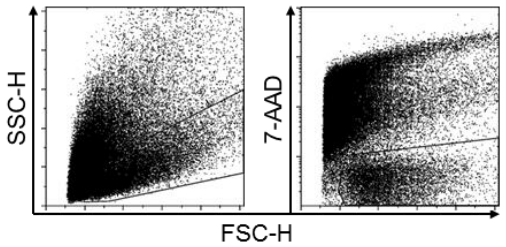
B10.D2 splenocytes prepared in **Experimental data 2** were stained with two reagents (I-A^d $OVA_{323-339}$ Tetramer-PE and anti-TCR DO11.10-FITC).

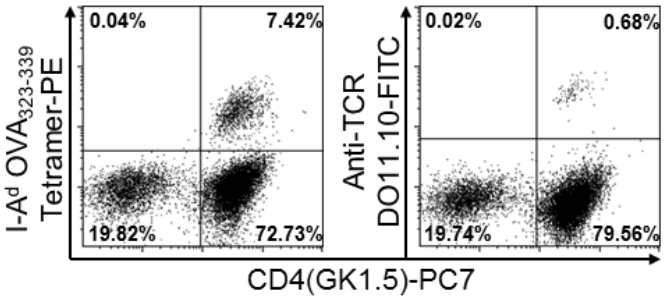
Protocols

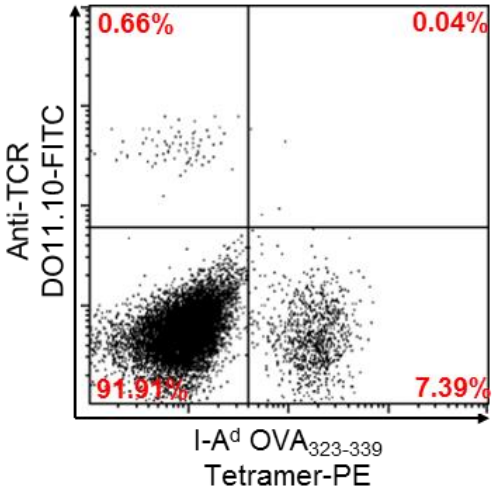
1. Aliquots of the splenocytes prepared in **Experimental data 2** should be re-suspended at a concentration of 2 x 10^7 cells/mL. 50 µL of sample is required for each T-Select MHC Tetramer determination.
2. Add 10 µL of Clear Back (human FcR blocking reagent) to each 12 x 75 mm test tube.
3. Add 50 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 following reagents together and vortex gently.
   - I-A^d $OVA_{323-339}$ Tetramer-PE
   - Anti-TCR DO11.10-FITC
6. Incubate for 60 minutes at 2-8°C protected from light.
7. Add 10 µL anti-mouse CD4 (GK1.5)-PE/Cy7 (PC7) antibody and vortex gently.
8. Incubate for 20 minutes at 2-8°C protected from light.
9. Add 1 mL of 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 FCM buffer (2% FCS/0.09% NaN_3/PBS).

Results 3

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 separated cells by quadrant is shown as a percentage of gated cells (R1 and R2).







The I-A^d $OVA_{323-339}$ Tetramer-positive T cells are different population from anti-TCR DO11.10 antibody-positive T cell population.