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Not for use in diagnostic procedures.**

MBL

T-Select MHC Class II Mouse Tetramer

I-A^b FMLV$_{123-141}$ Tetramer-PE (20 tests)

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^b and epitope peptide derived from envelope (gp70) protein of Friend murine leukemia virus (FMLV, F-MuLV), and it can detect an I-A^b-restricted FMLV$_{123-141}$-specific CD4$^+$ T cells. FMLV$_{123-141}$ epitope (H19-Env) is defined as immunodominant I-A^b restricted epitope and conserved in retrovirus that includes Moloney murine sarcoma virus (MoMSV). Instead of HIV, FMLV infection model has been a useful tool in analysis of T cells response to retrovirus infection and MuLV-induced tumors.

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.

Allele: I-A^b

Peptide Sequence: FMLV$_{123-141}$ peptide "EPLTSLTPRCNTAWNRLKL" derived from envelope protein of FMLV (FMLV, 123-141 aa)

Storage Conditions

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

Conjugates

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

Reagents

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.

Stability

This reagent is stable until the expiration date shown on the label under the recommended storage conditions.

Usage

This reagent is for use with standard flow cytometry methodologies.

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 liquid (SA-PE).

Mouse I-A Allele

MHC class II	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

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 and Rheumatism* **46**: 5-12 (2002)
4) Novak EJ, *et al*. *J. Clin. Ivest.* **104**: R63-R67 (1999)
5) Lyons AB and Doherty KV, *Current Protocols in Cytometry* **2**: 9.11.1-9.11.9 (1998)

References about FMLV$_{123-141}$

6) Iwashiro M, *et al*. *J. Virol*. **67**: 4533-4542 (1993)
7) Ossendorp F, *et al*. *J. Exp. Med.* **187**: 693-702 (1998)

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8) Schepers K, *et al. J. Immunol.* **169**: 3191-3199 (2002)
9) MacLeod M, *et al*. *J. Exp. Med*. **203**: 897-906 (2006)
10) Bayer W, *et al*. *J. Virol*. **84**: 1967-1976 (2010)
11) Lietz R, *et al*. *J. Virol.* **86**: 1706-1716 (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
- 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

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.
13. Store at 4°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.

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.

Related Products

Mouse MuLV Tetramers

TS-M507-1	H-2K^{b} MuLV p15E Tetramer-KSPWFTTL-PE
TS-M521-1	H-2L^{d} MuLV gp70 Tetramer-SPSYVYHQF-PE

Mouse class II Tetramers

TS-M705-1	I-A^{b} FMLV$_{123\text{-}141}$ Tetramer-PE
TS-M706-1	I-A^{b} E$\alpha_{52\text{-}68}$ Tetramer-PE
TS-M707-1	I-A^{b} ESAT-6$_{1\text{-}20}$ Tetramer-PE
TS-M710-1	I-A^{b} OVA$_{323\text{-}339}$ Tetramer-PE

Pick up Tetramers

TS-5001-1C	H-2K^{b} OVA Tetramer-SIINFEKL-PE
TS-5001-2C	H-2K^{b} OVA Tetramer-SIINFEKL-APC
TS-M008-1	H-2K^{b} Negative Tetramer-SIYRYYGL-PE
TS-M501-1	H-2K^{b} β-galactosidase Tetramer-DAPIYTNV-PE
TS-MCD-1	Mouse CD1d Tetramer–PE
TS-MCD-2	Mouse CD1d Tetramer–APC

Peptides

TS-5001-P	H-2K^{b} OVA peptide
TS-M507-P	H-2K^{b} MuLV p15E peptide
TS-M521-P	H-2L^{d} MuLV gp70 peptide
TS-M701-P	I-A^{b} HBc helper peptide
TS-M702-P	I-A^{d} Tetanus toxin p30 helper peptide
TS-M703-P	OVA$_{323\text{-}339}$ helper peptide
TS-M704-P	I-A^{b} MOG$_{35\text{-}55}$ peptide
TS-M707-P	I-A^{b} ESAT-6$_{1\text{-}20}$ peptide
TS-M708-P	I-A^{k} HEL peptide

Kit

AM-1005	IMMUNOCYTO Cytotoxity Detection Kit

Others

D341-4	mouse CD4-FITC (GK1.5)
D271-4	mouse CD8-FITC (KT15)
D271-A64	mouse CD8-Alexa Fluor® 647 (KT15)
732121	mouse CD16/32 (93)
K0221-3	anti-mouse TCR DO11.10 (KJ1.26)
K0221-5	PE labeled anti-mouse TCR DO11.10 (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)

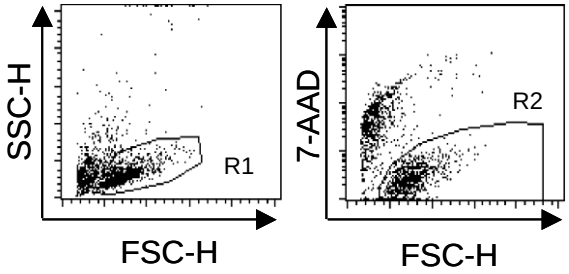
Please check our web site (http://ruo.mbl.co.jp) for up-to-date information on products and custom MHC Tetramers.

Experimental Data: C57BL/6 mice immunized with $FMLV_{123-141}$ peptide.

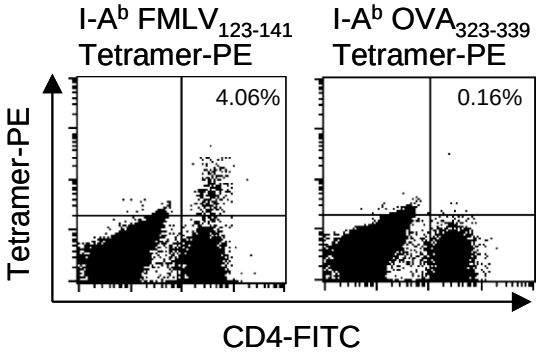
C57BL/6 mice were intraperitoneally immunized with 100 nmol $FMLV_{123-141}$ peptide (EPLTSLTPRCNTAW NRLKL) and 10 μg cholera toxin (MBL PN RK-01-511) in complete Freund's adjuvant. A second similar immunization was performed 10 days later. Splenocytes were prepared from the immunized mice 10 days after immunization. An aliquot of the splenocytes was stimulated with 1 μg/mL $FMLV_{123-141}$ peptide for 8 days in the presence of 50 U/mL recombinant human IL-2. The frozen stock of splenocytes on day 8 was thawed and stained with MHC class II Tetramer.

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



$FMLV_{123-141}$ peptide immunized mouse splenocytes (day8)



The I-A^b $FMLV_{123-141}$ Tetramer-positive $CD4^+$ T cells could be detected after *in vitro* stimulation for 8 days with the $FMLV_{123-141}$ peptide. Tetramer-positive $CD4^+$ T cells were not detected in the negative control (I-A^b $OVA_{323-339}$ Tetramer).

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