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

T-Select

Mouse CD1d Tetramer (α-GalCer loaded)

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Background

Natural Killer T (NKT) cells, a type of T cell that plays a significant role in immune response, produce a large quantity of INF-γ and IL-4 in response to glycolipids that presented by CD1d molecules. In recent years, NKT cells are reported to play a part in diabetes and tumor immunity. Therefore, a technology allowing quantitative measurement of CD1d-positive NKT cells would be a useful tool for immunology and clinical laboratory examinations.

The development of MHC Tetramer technology has provided a breakthrough in the ability to follow T cell populations defined by their antigen specificity. Tetramers have been used widely to obtain a detailed analysis of the distribution and frequency of conventional $CD4^+$ and $CD8^+$ antigen-specific T cells during a variety of immune responses. T-Select Mouse CD1d Tetramer is a reagent created by tetramerizing biotinylated mouse CD1d/β2m complexes with phycobiliprotein-labeled streptavidin. α-Galactosylceramide (α-GalCer), a glycosphingolipid originally isolated from marine sponges, appears to be presented by CD1d to activate both human and mouse NKT cells. T-Select Mouse CD1d Tetramer (α-GalCer loaded) is a highly specific reagent for detection of NKT cells. Measurement can be performed using isolated lymphocytes/monocytes.

Specificity

T-Select Mouse CD1d Tetramer (α-GalCer loaded) recognizes mouse NKT cells that bind specifically to CD1d / α-GalCer complex.

Reagents

500 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.

Storage Conditions

Store at 2 to 8°C. Do not freeze. Minimize exposure to light. The expiration date is indicated on the vial label.

Usage

This reagent is for use with standard flow cytometry methodologies.

Conjugates

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

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

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 for Products

1) Benlagha K, *et al. J Exp Med* **191**: 1895-1903 (2000)
2) Matsuda JL, *et al*. *J Exp Med* **192**: 741-754 (2000)
3) Sidobre S, and Mitchell K, *J Immunol Methods* **268**: 107-121 (2002)
4) Yoshiga Y, *et al*. *Clin Exp Immunol* **164**: 236–247 (2011)
5) Horikoshi M, *et al*. *PLoS One* **7**: e51215 (2012)
6) Nakamura T, *et al*. *J Control Release* **171**: 216–224 (2013)
7) Sekiya T, *et al*. *Nat Immunol* **14**: 230–237 (2013)
8) Adachi K, *et al*. *PLoS One* **9**: e96042 (2014)

Reagent Preparation

T-Select Mouse CD1d Tetramer (α-GalCer loaded) is loaded with α-GalCer and ready to use in cell staining. For use with ligands other than α-GalCer, we recommend empty CD1d tetramers (MBL, PN TS-MCD-1, TS-MCD-2).

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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
- Pipettors and disposable pipette tips
- Vortex mixer
- Centrifuge capable of 150 x g or 400 x g
- Aspirator
- PBS
- Red blood cell lysis reagent
- Anti-CD4 (Mouse) mAb-FITC (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 Whole Blood

1. Collect venous blood specimen according to established protocol into a blood collection tube using an appropriate anti-coagulant. If the mouse line that is being used is transgenic and the T cell receptor is specific for the peptide, 100 μL of whole blood should be adequate. If the blood specimen is not being derived from a transgenic line, you may require more than 100 μL in order to perform the rare event analysis.
2. To each 12 x 75 mm test tube add 10 μL of T-Select Mouse CD1d Tetramer (α-GalCer loaded)
3. Add 100 μL of whole blood into each test tube.
4. Vortex gently.
5. Incubate for 30-60 minutes at 2-8°C or room temperature (15-25°C) protected from light.
6. Add any additional antibodies (e.g. anti-CD4) and vortex gently.
7. Incubate for 30 minutes at 2-8°C protected from light.
8. Lyse red blood cells using commercially available reagents.
9. Prepare samples according to description of the package insert.
10. Analyze prepared samples by flow cytometry. If necessary, store the samples at 2-8°C protected from light for a maximum of 24 hours prior to analysis.

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 of cell suspension into each test tube (e.g. 1 x 10^6 cells per tube).
4. Incubate for 5 minutes at room temperature (15-25°C).
   Option:
   To reduce Fc receptor-mediated non-specific staining, preincubate cell suspension with proper quantity of anti-CD16/32 monoclonal antibody (e.g., clone 2.4G2 and 93) according to manufacturer's instructions.
5. Add 10 μL of T-Select Mouse CD1d Tetramer (α-GalCer loaded) into each test tube 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-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 8-9 in the **Procedure for Whole Blood** section.
   If red blood cell lysis is not necessary, continue to step 9 below.
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. Analyze prepared samples by flow cytometry. If necessary, store the samples at 2-8°C protected from light for a maximum of 24 hours prior to analysis.

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. If cell cultivation is needed, we recommend the use of heparin as an anti-coagulant.
B. 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.
C. T-Select Mouse CD1d Tetramer (TS-MCD-1 or TS-MCD-2, without α-GalCer) may be used as a negative control.
D. 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.
E. 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).
F. Cells do not require fixation prior to analysis if the stained cells are analyzed by flow cytometry within several hours.

Related Products

CD1d Tetramers

TS-MCD-1	Mouse CD1d Tetramer-PE
TS-MCD-2	Mouse CD1d Tetramer-APC
TS-MCG-1	Mouse CD1d Tetramer-PE (α-GalCer loaded)
TS-MCG-2	Mouse CD1d Tetramer-APC (α-GalCer loaded)
TS-HCD-1	Human CD1d Tetramer-PE
TS-HCD-2	Human CD1d Tetramer-APC
TS-HCG-1	Human CD1d Tetramer-PE (α-GalCer loaded)
TS-HCG-2	Human CD1d Tetramer-APC (α-GalCer loaded)

Antibodies for mouse NK and NKT cells

D341-4	Anti-CD4 (Mouse) mAb-FITC (GK1.5)
6604623	Anti-CD3 (Human) mAb-FITC (UCHT1)
D271-4	Anti-CD8 (Mouse) mAb-FITC (KT15)
D271-A64	Anti-CD8 (Mouse) mAb-Alexa Fluor® 647 (KT15)
D202-3	Anti-CD11b (Mouse) mAb (1C4)
D202-4	Anti-CD11b (Mouse) mAb-FITC (1C4)
D326-3	Anti-IL-17RB (Mouse) mAb (B5F6)
D327-3	Anti-IL-17RB (Mouse) mAb (3H8)

MHC class I mouse Tetramers

TB-5004-1	H-2K^b TRP2 Tetramer-SVYDFFVWL-PE
TS-M504-1	H-2D^b WT1$_{126-134}$ Tetramer-RMFPNAPYL-PE
TS-M505-1	H-2D^b human gp100 Tetramer-KVPRNQDWL-PE
TS-M546-1	H-2D^b gp100 Tetramer-EGSRNQDWL-PE
TS-M508-1	H-2D^b Influenza NP Tetramer-ASNENMETM-PE
TS-M528-1	H-2D^b Influenza PA Tetramer-SSLENFRAYV-PE
TS-M520-1	H-2K^d Influenza HA Tetramer-IYSTVASSL-PE
TB-M534-1	H-2K^d Influenza NP Tetramer-TYQRTRALV-PE
TB-5002-1	H-2D^b LCMV gp$_{33}$ Tetramer-KAVYNFATC-PE
TS-M512-1	H-2D^b LCMV gp$_{33}$ (C9M) Tetramer-KAVYNFATM-PE
TS-M513-1	H-2D^b LCMV NP$_{396}$ Tetramer-FQPQNGQFI-PE
TB-5010-1	H-2K^b LCMV gp$_{34-41}$ Tetramer-AVYNFATC-PE
TB-5007-1	H-2K^b HIV gag Tetramer-AMQMLKETI-PE
TB-5018-1	H-2D^b RSV Tetramer-NAITNAKII-PE
TS-M506-1	H-2K^d RSV Tetramer-SYIGSINNI-PE
TS-M507-1	H-2K^b MuLV p15E Tetramer-KSPWFTTL-PE
TS-M521-1	H-2L^d MuLV gp70 Tetramer-SPSYVYHQF-PE
TB-M537-1	H-2K^b HBV core Tetramer-MGLKFRQL-PE
TB-5008-1	H-2D^b HPV16 E7 Tetramer-RAHYNIVTF-PE
TB-5016-1	H-2D^b MoMSV Tetramer-(Abu)(Abu)L(Abu)LTVFL-PE
TB-5017-1	H-2D^b SIV gag Tetramer-AAVKNWMTQTL-PE
TS-5001-1C	H-2K^b OVA Tetramer-SIINFEKL-PE
TS-M008-1	H-2K^b Negative Tetramer-SIYRYYGL-PE
TS-M525-1	H-2K^d EGFP Tetramer-HYLSTQSAL-PE
TS-M501-1	H-2K^b β-galactosidase Tetramer-DAPIYTNV-PE
TS-M511-1	H-2L^d β-galactosidase Tetramer-TPHPARIGL-PE
TB-M552-1	H-2K^d IGRP Tetramer-VYLKTNVFL-PE
TB-M553-1	H-2K^d NRP-V7 Tetramer-KYNKANVFL-PE

MHC class II mouse Tetramers

TS-M704-1	I-A^b MOG$_{35-55}$ Tetramer-PE
TS-M705-1	I-A^b FMLV$_{123-141}$ Tetramer-PE
TS-M706-1	I-A^b Eα$_{52-68}$ Tetramer-PE
TS-M707-1	I-A^b ESAT-6$_{1-20}$ Tetramer-PE
TS-M710-1	I-A^b OVA$_{323-339}$ Tetramer-PE
TS-M703-1	I-A^d OVA$_{323-339}$ Tetramer-PE
TS-M722-1	I-A^b mouse 2W1S Tetramer-PE
TS-M720-1	I-A^d human CLIP$_{103-117}$ Tetramer-PE
TS-M715-1	I-A^b human CLIP$_{103-117}$ Tetramer-PE
TS-M716-1	I-A^b Influenza NP$_{311-325}$ Tetramer-PE
TS-M723-1	I-A^b *T. gondii* CD4Ag28m$_{605-619}$ Tetramer-PE
TS-M727-1	I-A^{g7} BDC2.5 mimotope Tetramer-PE
TS-M717-1	I-A^{g7} human CLIP$_{103-117}$ Tetramer-PE
TS-M718-1	I-A^{g7} chicken HEL$_{11-25}$ Tetramer-PE

Kits

AM-1005	IMMUNOCYTO Cytotoxity Detection Kit
TB-7400-K1	QuickSwitch™ Quant H-2K^b Tetramer Kit-PE
TB-7401-K1	QuickSwitch™ H-2K^b Tetramer Kit-PE

Others

K0221-3	Anti-TCR DO11.10 (Mouse) mAb (KJ1.26)
K0221-5	Anti-TCR DO11.10 (Mouse) mAb-PE (KJ1.26)
K0222-3	Anti-TCR 3DT-52.5 (Mouse) mAb (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

Procedure

1. Prepare mice splenocytes hemolyzed with ACK lysis buffer, and wash in FCM buffer (2% FCS/0.05% NaN_3/PBS) in each test tube.
2. Add 1 mL of FCM buffer, and centrifuge at 400 x g for 5 minutes.
3. Aspirate the supernatant carefully. Add 10 μL of Clear Back (MBL, PN MTG-001) and 30 μL of FCM buffer. Incubate for 5 minutes at room temperature (15-25°C).
4. Add 10 μL of T-Select Mouse CD1d Tetramer (α-GalCer loaded) or T-Select Mouse CD1d Tetramer (TS-MCD-2, without α-GalCer) to each test tube and mix well. Incubate the cells for 30 minutes at 2-8°C.
5. Add 10 μL of anti-mouse CD4-FITC (clone GK1.5, MBL, PN D341-4) to each test tube and mix well. Incubate for 30 minutes at 2-8°C.
6. Add 1 mL of FCM buffer, and centrifuge at 400 x g for 5 minutes.
7. Aspirate the supernatant carefully. Suspend the cells with 400 μL of FCM buffer.
8. Add 5 μL of 7-AAD (MBL, PN A07704) for the exclusion of nonviable cells in flow cytometric assays.
9. Analyze prepared samples by flow cytometry.

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 frequencies of Mouse CD1d Tetramer+ CD4+ cells are shown as a percentage in BALB/c (Figure 1) or in C57BL/6 splenocytes (Figure 2).

α-GalCer / Mouse CD1d Tetramer-positive CD4+ cells were detected in both BALB/c and C57BL/6 mice splenocytes.

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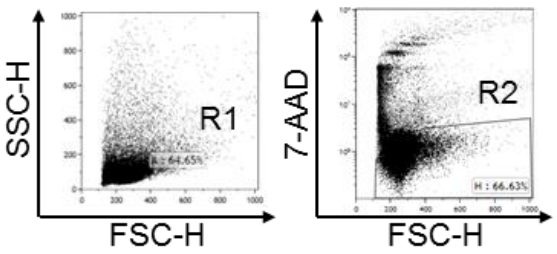

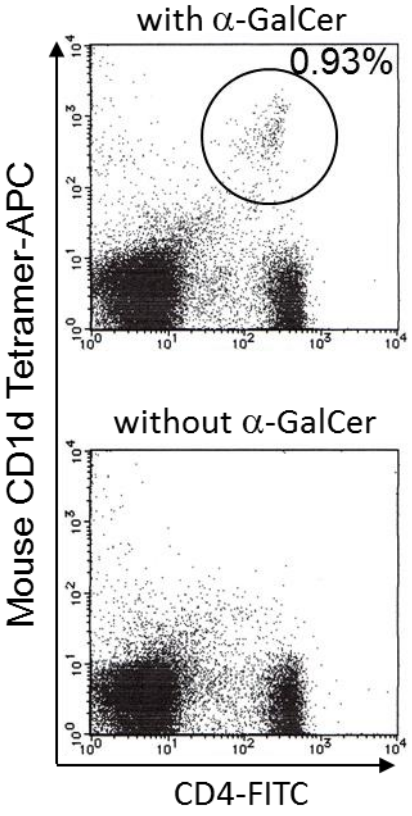

Figure 1 BALB/c splenocytes

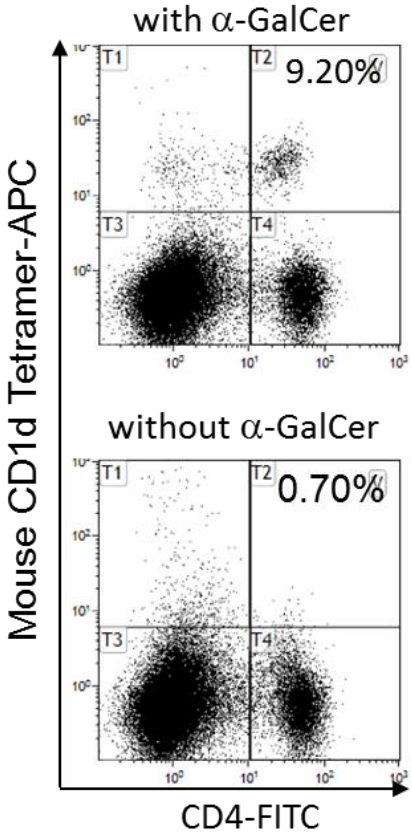

Figure 2 C57BL/6 splenocytes