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

T-Select

Human 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, produces 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 Human CD1d Tetramer is a reagent created by tetramerizing biotinylated human 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 Human 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 Human CD1d Tetramer (α-GalCer loaded) recognizes human 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-HCG-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-HCG-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) Matsuda JL, *et al*. *J*. *Exp*. *Med*. **192**: 741-754 (2000)
2) Karadimitris A, *et al*. *PNAS* **98**: 3294-3298 (2001)
3) Kita H, *et al*. *Gastroenterology* **123**: 1031-1043 (2002)
4) Sidobre S, and Kronenberg M, *J*. *Immunol*. *Methods* **268**: 107-121 (2002)
5) Wu D, *et al*. *PNAS* **102**: 1351-1356 (2005)
6) Li D, *et al*. *J*. *Immunol*. **182**: 1033-1040 (2009)

Reagent Preparation

T-Select Human 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-HCD-1, TS-HCD-2).

Cell Staining Procedure

1. Prepare single cell suspension from anticoagulated human peripheral blood according to the standard procedure. For staining, suspend the cells in FCM buffer (2% FCS/0.05% NaN_3/PBS) at a concentration of up to 1 x 10^7 cells/mL.
2. Add 100 μL of cell suspension to each test tube and centrifuge it at 400 x g for 5 minutes. Aspirate the supernatant.
3. Add 10 μL of Clear Back (human Fc receptor blocking reagent, MBL, PN MTG-001) and 30 μL

of FCM buffer to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.

4. Add 10 μL of T-Select Human CD1d Tetramer (α-GalCer loaded) to each test tube and mix well. Incubate the cells in the dark for 20 minutes at 2-8ºC.
5. Add 1 mL of FCM buffer.
6. Centrifuge at 400 x g for 5 minutes. Aspirate the supernatant. Repeat the same operation for three times.
7. Suspend the pellet with 500 μL of FCM buffer. Analyze it immediately, or suspend it with 0.5% paraformaldehyde/PBS, and store the sample in dark room at 2-8ºC. Be sure to analyze it within 24 hours.

* To add antibodies such as CD3, add them at the step 4.

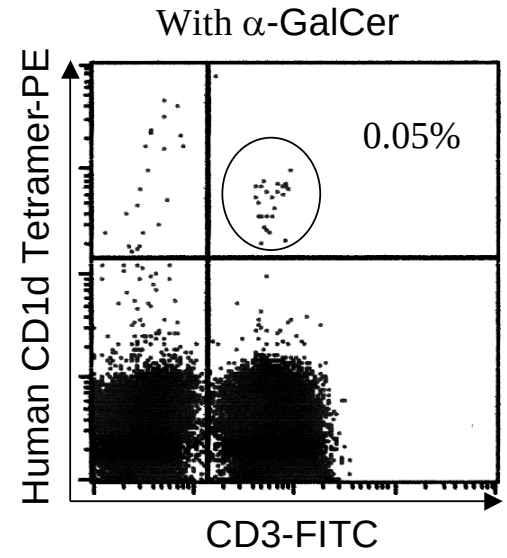
Consideration

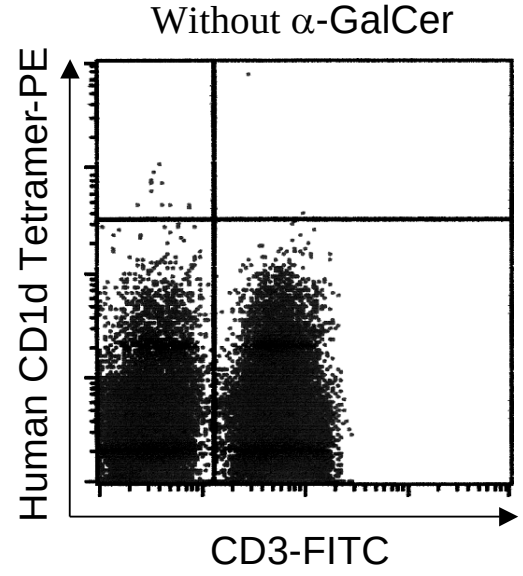
A. If the blood erythrocyte remains in the cell sample, we recommend hemolyzing them. If the blood erythrocyte still remains after being hemolyzed, we recommend staining the cells with anti-CD45 antibody simultaneously and analyzing the result with lymphocyte gating.
B. We recommend using Clear Back (MBL, PN MTG-001) to reduce nonspecific staining of cells by endocytosis in the macrophages.
C. For staining of *in vitro* cultured lymphocyte, we recommend staining with 7-AAD for exclusion of dead cells and non-viable cells.
D. Paraformaldehyde fixation of cells is not needed if the cells are analyzed within a couple of hours after staining.

Precautions

1. The reagent contains 0.09% of 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. Samples and all materials coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
4. Minimize exposure of reagents to light during storage or incubation.
5. Recommended cell survival rate of venous blood specimen is > 90%.
6. To obtain appropriate result with whole blood, we recommend to keeping the test sample in a blood collection tube at room temperature and turning upside-down repeatedly just before staining. Do not use cold test blood in order to have appropriate result.
7. Do not incubate the cells for a long time with the hemolysis reagent. The long incubation results in the disruption of leukocyte.
8. Erythrocyte of abnormal test blood, such as nucleated erythrocyte, blood of abnormal hemoglobin disease, cannot be hemolyzed well. In such case, the unhemolyzed erythrocyte is improperly counted as a leukocyte. This improper count results in increasing the number of leukocyte and decreasing the number of the positive rate of NKT cells.

Example of human PBMC staining using T-Select Human CD1d Tetramer-PE with (upper) or without α-GalCer (lower)





Related Products

CD1d Tetramers

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)
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)

Antibodies for NKT

IM-1589	Anti-TCR Vα 24 (Human) mAb-FITC (C15)
IM-2283	Anti-TCR Vα 24 (Human) mAb-PE (C15)
IM-1586	Anti-TCR Vβ 11 (Human) mAb-FITC (C21)
IM-2290	Anti-TCR Vβ 11 (Human) mAb-PE (C21)
IM-1280	Anti-CD3 (Human) mAb (UCHT1) (100 tests)
IM-1304	Anti-CD3 (Human) mAb (UCHT1) (200 μg)
A07746	Anti-CD3 (Human) mAb-FITC (UCHT1)
A07747	Anti-CD3 (Human) mAb-PE (UCHT1)
A07749	Anti-CD3 (Human) mAb-PC5 (UCHT1)
IM-2467	Anti-CD3 (Human) mAb-APC (UCHT1)
IM-0398	Anti-CD4 (Human) mAb (13B8.2)
A07750	Anti-CD4 (Human) mAb-FITC (13B8.2)
A07751	Anti-CD4 (Human) mAb-PE (13B8.2)
A07752	Anti-CD4 (Human) mAb-PC5 (13B8.2)
IM-2468	Anti-CD4 (Human) mAb-APC (13B8.2)
6603861	Anti-CD8α (Human) mAb-FITC (T8)
A07757	Anti-CD8α (Human) mAb-PE (B9.11)
6607011	Anti-CD8α (Human) mAb-PC5 (T8)
IM-2469	Anti-CD8α (Human) mAb-APC (B9.11)
A07782	Anti-CD45 (Human) mAb-FITC (J.33)
A07783	Anti-CD45 (Human) mAb-PE (J.33)
A07785	Anti-CD45 (Human) mAb-PC5 (J.33)
IM-2473	Anti-CD45 (Human) mAb-APC (J.33)
IM-1943	Anti-CD69 (VEA) (Human) mAb-PE (TP1.55.3)
IM-2656	Anti-CD69 (VEA) (Human) mAb-PC5 (TP1.55.3)
IM-2276	Anti-CD94 (Human) mAb-PE (HP-3B1)
K0061-3	Anti-CD161 (Human) mAb (HP-3G10)
K0061-4	Anti-CD161 (Human) mAb-FITC (HP-3G10)

Antibodies for NK

6604894	Anti-CD16 (Human) mAb-FITC (3G8)
A07766	Anti-CD16 (Human) mAb-PE (3G8)
A07767	Anti-CD16 (Human) mAb-PC5 (3G8)
6607118	Anti-CD16 (Human) mAb-PC7 (3G8)
A07788	Anti-CD56 (Human) mAb-PE (N901)
A07789	Anti-CD56 (Human) mAb-PC5 (N901)
A21692	Anti-CD56 (Human) mAb-PC7 (N901)
IM-2474	Anti-CD56 (Human) mAb-APC (N901)
IM-0466	Anti-CD57 (Human) mAb-FITC (NC1)

Kits

AM-1005	IMMUNOCYTO Cytotoxity Detection Kit
TB-7300-K1	QuickSwitch™ Quant HLA-A*02:01 Tetramer Kit-PE
TB-7301-K1	QuickSwitch™ HLA-A*02:01 Tetramer Kit-PE
TB-7302-K1	QuickSwitch™ Quant HLA-A*24:02 Tetramer Kit-PE
TB-7303-K1	QuickSwitch™ HLA-A*24:02 Tetramer Kit-PE

Others

MTG-001	Clear Back (Human FcR blocking reagent)
A07704	7-AAD Viability Dye
IM-1400	OptiLyse B
A11895	OptiLyse C

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