T-Select

Mouse CD1d Tetramer

使用は研究用に限ります。診断目的には使用しないでください。
本試薬にα-GalCerは含まれておりません。

容量:
<table>
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<th>Code no</th>
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<td>TS-MCD-1S</td>
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背景:
CD1d分子は、β2-マイクログロビリン（β2m）と非共有結合した細胞膜タンパク質で、ヒト、マウス間でホモロジーが非常に高いことが知られています。CD1d分子は、海綿から分離・合成された糖脂質であるα-galactosylceramide（α-GalCer）を認識することが知られています。CD1d分子は、海綿から分離・合成された糖脂質であるα-galactosylceramide（α-GalCer）を認識することで、CD1d拘束性のNKT細胞をFACSで検出することができ、診断、治療にも関与していることが報告されています。免疫系用であると考えられます。

術は、免疫学だけでなく、臨床研究においても極めて有用であると考えられています。

T-Select Mouse CD1d Tetramerは、mouse CD1dとβ2mの複合体を蛍光標識させたストレプトアビシンにより4量体化した試薬です。本試薬にα-GalCerを結合させることで、CD1d拘束性のNKT細胞をFACSで検出できることが知られています。

特異性: T-Select Mouse CD1d Tetramerは、CD1d分子とα-GalCer複合体に特異的に結合するマウスNKT細胞を認識します。

保存法: 2-8℃で遮光保存してください。凍結は絶対にしないでください。製品有効期限は、チューブに貼られているラベルをご確認ください。

標識物:
TS-MCD-1 (S): Streptavidin-Phycoerythrin (SA-PE)
励起波長: 486-580 nm
蛍光波長: 586-590 nm

TS-MCD-2: Streptavidin-Allophycocyanin (SA-APC)
励起波長: 633-635 nm
蛍光波長: 660-680 nm

試薬の調製: T-Select Mouse CD1d Tetramerには、α-GalCerは含まれていません。従いまして、以下の手順で、α-GalCerを結合後、使用してください。
1. α-GalCerを1 mg/mLの濃度となるように(pyridineで)溶解します。これをstock溶液として、0.05%Tween-20/0.9%NaClで200 µg/mLの濃度となるように希釈します。
2. 5 µLの希釈したα-GalCerを100 µLのT-Select Mouse CD1d Tetramerに加えて、全体をよくよく洗浄して保存してください。皮膚や目に入った場合には十分量の水で洗い流してください。
3. 静置後、そのままの状態で4℃に保存します。

染色方法:
1. 定められた手順に従い、脾臓、胸臓、リンパ節、末梢血などで細胞懸濁液を調製します。染色には約3 x 10^6 cells/mLの濃度にて、細胞をFCM buffer（2%BSA/0.05%NaN3/PBS）で再懸濁します。
2. 各試験管に100 µLの細胞懸濁液を添加します。適量のanti-FcγR antibody（MBL code no. 732121）を加え、60分間、暗所で反応させてからFACSで検出するとよいです。
加えて、4℃で15分間インキュベートします。
3. FCM bufferを1mL加えます。
4. 400xgで5分間遠心分離します。上澄みをアスピレートします。同様の操作を2回繰り返します。
5. 100μLのFCM bufferで再懸濁します。
6. 10μLのT-Select Mouse CD1d Tetramerを各試験管に加え、よく混ぜます。暗所温室にて30分インキュベートします。
7. 1mLのFCM bufferを加えます。
8. 400xgで5分間遠心分離します。上澄みをアスピレートします。同様の操作を3回繰り返します。
9. ペレットを500μLのPBSで再懸濁し、フローサイトメーターで解析します。直ちに分析しない場合は、サンプルを0.5%パラフォルムアルデヒド/PBSにて再懸濁して暗所4℃で保管し、24時間以内に分析してください。
*CD4などの抗体を追加する場合はステップ6で添加してください。

染色の注意点:
A. 染色する細胞集団に赤血球の残存が認められる場合は、溶血処理を行ってください。溶血処理後も赤血球の混入が認められる場合はCD4を同時染色し、リンパ球ゲートにて解析してください。
B. Clear Back（MBL code no. MTG-001）を用いることで、マクロファージなどのエンドサイトーシスによる非特異的染色を抑制する効果が期待されます。
C. 培養したリンパ球を染色する場合は、7-AADを用いて死細胞を染色し、解析ゲート内から除去してください。
D. 染色後、数時間以内に解析する予定でしたら、パラフォルムアルデヒドによる固定処理は必要ありません。

一般的な注意事項:
1. 検体、サンプル、およびそれらと接触する全ての材料は感染の可能性を持つものとして、取り扱いには十分注意してください。
2. 保管もしくは反応中、試薬に光をあてないようにご注意ください。
3. 試薬の細菌感染を避けてください。誤った結果が出ることがあります。
4. 当試薬の取り扱いにはGood Laboratory Practice（GLP）をご参照ください。
5. 全血にて最適な結果を得るため、検体は採血管にて温室で保存し、染色操作直前にも倒立攪拌してください。冷蔵検体では異常な結果が出ることがありますので使用しないでください。
6. 細胞を溶血試薬と長時間反応させないでください。白血球の破壊や目的細胞損失の原因となります。
7. 有核赤血球、異常タンパク濃度を有する検体、もしくは異常色性症では、必ずしも全ての赤血球が溶血されないことがあります。こうした場合、溶血されない赤血球が白血球としてカウントされることで、陽性率の低下をもたらすことがあります。
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 streptavidin labeled phycobiliprotein. 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. α-GalCer loaded T-Select Mouse CD1d Tetramer is a highly specific reagent for detection of NKT cells. Measurement can be performed using isolated lymphocytes/monocytes.

Specificity:

T-Select Mouse CD1d Tetramer recognizes mouse NKT cells that bind specifically with CD1d molecule and α-GalCer complex.

Storage:

Store protected from light at 2-8°C. Do not freeze.

Fluorescent labeling:

Phycoerythrin
Excitation wavelength: 486 - 580 nm
Fluorescent wavelength: 586 - 590 nm

Allophycocyanin
Excitation wavelength: 633 - 635 nm
Fluorescent wavelength: 660 - 680 nm

Formulation:

The reagent is dissolved in aqueous buffer that contains 10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5 mM EDTA, 0.2% BSA and 0.09% NaN₃. *Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

Stability:

The product is stable for at least 2 year when stored as recommended. If the reagent shows physical change (usually it is a slightly pinkish or blue liquid), do not use the reagent because it may be degraded.

Preparation of reagent:

T-Select Mouse CD1d Tetramer-PE does not contain α-GalCer. Please combine with α-GalCer before use according to the procedure described below.

1. Dissolve α-GalCer in pyridine to make a concentration of 1 mg/mL. Use this as a stock solution, and dilute it with 0.5% Tween-20, 0.9% NaCl to make a concentration of 200 μg/mL.
2. Add 5 μL of diluted α-GalCer to 100 μL of T-Select Mouse CD1d Tetramer-PE, and pipette the whole solution slowly. Incubate it at room temperature (20-30°C) or 37°C for 12 to 18 hours. Minimize exposure to light.
3. After that, store the reagent as is at 4°C.

Cell staining procedure:

1. Prepare single cell suspension from spleen, thymus, lymph node and peripheral blood according to the standard procedure. For staining, suspend the cells in FCM buffer (2% BSA/0.05% NaNO₃/PBS) at a concentration of up to 3 x 10⁷ cells/mL.
2. Add 100 μL of cell suspension to each test tube. Add proper quantity of anti-FcγR antibody (MBL code no. 732121), and incubate the cells for 15 minutes at 4°C.
3. Add 1 mL of FCM buffer.
4. Centrifuge at 400 x g for 5 minutes. Aspirate the supernatant. Repeat the same operation twice.
5. Add 200 μL of cell suspension to each test tube and mix well. Incubate the cells in the dark for 30 minutes at room temperature.
6. Add 1 mL of FCM buffer.
7. Add 1 mL of FCM buffer.
8. Centrifuge at 400 x g for 5 minutes. Aspirate the supernatant. Repeat the same operation for three times.
9. Add 1 mL of FCM buffer.
10. Centrifuge at 400 x g for 5 minutes. Aspirate the supernatant. Repeat the same operation for three times.
11. Add 1 mL of FCM buffer.

* To add antibodies such as CD4, add them at the step 6.

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 code no. 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. Avoid microbial contamination of reagents or incorrect results might occur.
6. Use Good Laboratory Practice (GLP) when handling this reagent.
7. 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.
8. Do not incubate the cells for a long time with the hemolysis reagent. The long incubation results in the disruption of leukocyte.
9. Erythrocyte of abnormal test blood, such as nucleated erythrocyte, blood of abnormal hemoglobin disease, can not 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 Balb/c mouse splenocyte staining using T-Select Mouse CD1d Tetramer-PE with (upper) or without α-GalCer (lower)
References:

Related Products:

**CD1d Tetramers**
- TS-MCD-1 mouse CD1d Tetramer-PE (50 tests)
- TS-MCD-1S mouse CD1d Tetramer-PE (10 tests)
- TS-MCD-2 mouse CD1d Tetramer-APC (50 tests)
- TS-HCD-2 human CD1d Tetramer-APC (50 tests)
- TS-MCD-1S mouse CD1d Tetramer-PE (10 tests)
- TS-HCD-2 human CD1d Tetramer-APC (50 tests)

**T-Select Mouse Tetramers**

**Virus**
- TS-M502-1 H-2D^d Fluorescence NP Tetramer-ASNYEMDTM-PE
- TS-M508-1 H-2D^d Fluorescence NP Tetramer-ASNYEMDTM-PE
- TS-M520-1 H-2K^k Fluorescence HA Tetramer-IVTAVASL-PE
- TS-5002-1 H-2D^d LCMV gp33 Tetramer-KAVYNFATC-PE
- TS-5002-2 H-2D^d LCMV gp33 Tetramer-KAVYNFATC-APC
- TS-M512-1 H-2D^d LCMV gp33 (19M) Tetramer-KAVYNFATC-PE
- TS-M513-1 H-2D^d LCMV NP396 Tetramer-FOPONQGQFI-PE
- TS-M514-1 H-2L^d LCMV NP118 Tetramer-RPOASGQYMP-PE
- TS-M516-1 H-2D^d HIV P18-110 Tetramer-RGPGRGAVT-PE
- TS-5007-1 H-2K^k HIV gag Tetramer-AMQMLKETI-PE
- TS-5007-2 H-2K^k HIV gag Tetramer-AMQMLKETI-APC
- TS-M506-1 H-2K^k RSV Tetramer-SYGISINNI-PE
- TS-M506-2 H-2K^k RSV Tetramer-SYGISINNI-APC
- TS-M507-1 H-2K^k MuLV p15E Tetramer-KSPNFTTL-PE
- TS-M521-1 H-2L^d MuLV gp70 Tetramer-SPSYVYHGF-PE
- TS-M509-1 H-2K^k SeV Tetramer-FAPGYNYPAL-PE
- TS-M510-1 H-2L^d MCMV IE1 Tetramer-PYPHPMTNL-PE
- TS-M522-1 H-2L^d HBAg Tetramer-IPGSLDSWNTSL-PE
- TS-M523-1 H-2K^k HSV-1 gB Tetramer-SSIEFARL-PE

**Foreign antigens**
- TS-5001-1 H-2K^k OVA Tetramer-SINFHKL-PE
- TS-5001-2 H-2K^k OVA Tetramer-SINFHKL-APC
- TS-M503-1 H-2K^k Listeria LLO Tetramer-GKYDGMNIEY-PE
- TS-M515-1 H-2K^k malaria Tetramer-SYISPPAEKI-PE
- TS-M517-1 H-2D^d BCG MPT51 Tetramer-GPHAVVYLL-PE
- TS-M501-1 H-2K^k β-galactosidase Tetramer-DAPYTNV-PE
- TS-M511-1 H-2L^d β-galactosidase Tetramer-TPHARGL-PE

Other
- TS-M508-1 H-2K^k Negative Tetramer-SIYRRYGL-PE

**T-Select Peptides**
- TS-5001-P H-2K^k OVA peptide
- TS-M501-P H-2K^k β-galactosidase peptide
- TS-M502-P H-2D^d influenza NP peptide
- TS-M503-P H-2K^k Listeria LLO peptide
- TS-M505-P H-2D^d human gp100 peptide
- TS-M506-P H-2K^k RSV peptide
- TS-M507-P H-2K^k MuLV peptide
- TS-M508-P H-2D^d influenza NP peptide
- TS-M509-P H-2K^k SeV peptide
- TS-M510-P H-2L^d MCMV IE1 peptide
- TS-M511-P H-2L^d β-galactosidase peptide
- TS-M512-P H-2D^d LCMV gp33 (19M) peptide
- TS-M513-P H-2D^d LCMV NP396 peptide
- TS-M514-P H-2L^d LCMV NP118 peptide
- TS-M515-P H-2K^k malaria peptide
- TS-M516-P H-2D^d HIV P18-110 peptide
- TS-M517-P H-2D^d BCG MPT51 peptide
- TS-M518-P H-2D^d OEA peptide
- TS-M519-P H-2L^d P815 peptide
- TS-M520-P H-2K^k influenza HA peptide
- TS-M521-P H-2L^d MuLV gp70 peptide
- TS-M522-P H-2L^d HBsAg peptide
- TS-M523-P H-2K^k HSV-1 gB peptide
- TS-M524-P H-2D^d HU ty peptide
- TS-M525-P H-2K^k EGFP peptide
- TS-M526-P H-2K^k HER2 peptide
- TS-M527-P H-2D^d Fluorescence NP peptide
- TS-M526-P H-2D^d influenza PA peptide
- TS-M529-P H-2K^k VSV NP peptide
- TS-M530-P H-2D^d polymavirus MT peptide
- TS-M531-P H-2D^d HTLV-1 Tax38-46 peptide
- TS-M508-P H-2K^k SIV peptide
- TS-M701-P I-AY HBe helper peptide
- TS-M702-P I-AY Tetanus toxoid p30 helper peptide
- TS-M703-P I-AY OVA 323-339 helper peptide
- TS-M704-P I-AY MOG peptide
- TS-M707-P I-AY ESAT-6 peptide
- TS-M708-P I-AY HEL peptide

**Kit**
- AM-1005 IMMUNOCYTO Cytotoxicity Detection Kit

**Antibody for mouse NK and NKT cells**
- IM-2767 mouse CD3 (clone KT3)
- IM-2770 mouse CD3-Biotin (clone KT3)
- IM-2788 mouse CD3-FITC (clone KT3)
- IM-2799 mouse CD3-PE (clone KT3)

- 731998 mouse CD4 (clone GK1.5)
- 732001 mouse CD4-Biotin (clone GK1.5)
- 732002 mouse CD4-FITC (clone GK1.5)
- 732004 mouse CD4-PE (clone GK1.5)
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<td>mouse CD16/32 (93)</td>
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<td>K0221-3</td>
<td>anti-mouse TCR DO11.10 (KJ1.26)</td>
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<td>K0221-5</td>
<td>PE labeled anti-mouse TCR DO11.10 (KJ1.26)</td>
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<td>K0222-3</td>
<td>anti-mouse TCR 3DT–52.5 (KJ12.98)</td>
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<td>A07704</td>
<td>7–AAD Viability Dye</td>
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<tr>
<td>MTG-001</td>
<td>Clear Back (Human FcR blocking reagent)</td>
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