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

MBL
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T-Select
Human MR1 Tetramer v2 (50 tests)

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These reagents do NOT include MR1 ligand.

Background

MHC class I-related protein (MR1) is classified as a non-classical class I molecule and has the ability to activate mucosal-associated invariant T cells (MAIT cells) by presenting vitamin B metabolites to them. Similar to MHC class I molecules, MR1 associates with β2-microglobulin (β2m), and like CD1, MR1 is a monomorphic molecule. While MHC class I molecules bind to peptides and CD1d binds to glycolipids, MR1 presents microbial vitamin B metabolites to MAIT cells. In addition, some other drugs and drug-like molecules are reported to be presented by MR1.

Human MAIT cells are abundant and localize in the mucosa, liver, and peripheral blood. MAIT cells are proposed to act as innate T cells and primarily respond to bacterial and mycotic infections. They are also reported to be associated with autoimmune diseases, e.g., systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and type 1 diabetes.

T-Select Human MR1 Tetramer is a reagent prepared by tetramerizing biotinylated human MR1/β2m complexes with phycobiliprotein-labeled streptavidin.

T-Select Human MR1 Tetramer allows the development and phenotypic characterization of MAIT cells and helps reveal the role of MAIT cells in health and disease.

Specificity

T-Select Human MR1 Tetramer recognizes human MAIT cells that bind specifically to the MR1/MR1-ligand 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 2-8°C. Do not freeze. Minimize exposure to light. The expiration date is indicated on the vial label.

Usage

These reagents are for use with standard flow cytometry methodologies.

Conjugates

TS-HMRV2-1
Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-HMRV2-2
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 if this occurs. The reagent normally appears as a clear, colorless to pink (SA-PE) or light blue (SA-APC) solution.

References for Products

1) Dusseaux M, *et al. Blood* **117**:1250-1259 (2011)
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3) Reantragoon R, *et al. J Exp Med* **210**:2305-2320 (2013)
4) Corbett AJ, *et al. Nature* **509**:61-365 (2014)
5) Eckle SB, *et al. J Exp Med* **211**:1585-1600 (2014)
6) Howson LJ, *et al. Front Immunol* **6**:303 (2015)
7) Mondot S, *et al. Immunogenetics*.**68**:537-548 (2016)
8) Keller AN, *et al. Nat Immunol* **18**:402-411 (2017)
9) Keller AN, *et al. Curr Opin Immunol* **46**:66-74 (2017)
10) Greene JM, *et al. Mucosal Immunol* **10**:802-813 (2017)
11) Kjer-nielsen L, *et al. Immunol Cell Biol* **6**:573-587 (2018)
12) Chiba A, *et al. Front Immunol* **9**:1333 (2018)

Related Products

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

Reagent Preparation

T-Select Human MR1 Tetramers do NOT include the MR1 ligand. Please combine with the MR1 ligand before use. Below is an example of the procedure for loading typical ligands.

[5-OP-RU]

It is reported that 5-(2-oxopropylideneamino)-6-Dribitylaminouracil (5-OP-RU) binds strongly to MR1, whereas 5-OP-RU is expected to be far too unstable in water. 5-OP-RU is generated by reacting 5-amino-6-D-ribitylaminouracil (5-A-RU) with methylglyoxal as follows:

1. Dissolve 5-A-RU dihydrochloride in water to a concentration of 2.76 mg/mL.
2. Mix 1 μL methylglyoxal and 406 μL water (MG solution).
3. Mix the 5-A-RU solution and the MG solution in the ratio of 1:1.
4. Add 0.72 μL of the mixture from step 3 to 100 μL of T-Select Human MR1 Tetramer and mix gently but thoroughly by pipetting. Incubate at 2-8°C for 12 to 18 hours. Minimize exposure to light.
5. After that, store the reagent at 2-8°C.

[Ac-6-FP, 6-FP]

6-formylpterin (6-FP), which is a photodegradation product of folic acid, binds to MR1, but it is reported that 6-FP does not activate MAIT cells. In addition, acetyl-6-formylpterin, a synthetic analogue of 6-FP is more potent than 6-FP in inhibiting the activation of MAIT cells. Therefore, 6-FP or the Ac-6-FP loaded MR1 tetramer can be used as a negative control.

1. Dissolve Ac-6-FP in water supplemented with 10 mM NaOH to a concentration of 2.0 mg/mL.
2. Add 0.5 μL of the Ac-6-FP solution to 100 μL of T-Select Human MR1 Tetramer and mix gently but thoroughly by pipetting. Incubate at 2-8°C for 12 to 18 hours. Minimize exposure to light.
3. After that, store the reagent at 2-8°C.

Cell Staining Procedure

1. Prepare a 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 up to 2-10 x 10^5 cells/tube.
2. Add 5 μL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
3. Add 10 μL of T-Select Human MR1 Tetramer loaded with the MR1 ligand and dilute to 50 μL with FCM buffer. Mix well and incubate the cells in the dark for 30-60 minutes at 2-8°C.
4. Add 500 μL of FCM buffer and centrifuge at 400 × g for 3 minutes. Aspirate the supernatant.
5. Add 1 μL of Anti-PE mAb (or Anti-APC mAb) (MBL, code no. M240-3, M241-3) as an enhancer and dilute to 50 μL with FCM buffer. Mix well and incubate the cells in the dark for 20 minutes at 2-8°C.
6. Wash the cells as step 4.
7. Add 5 μL of Anti-CD3 antibody and dilute to 50 μL with FCM buffer. Mix well and incubate the cells in the dark for 30 minutes at 2-8°C.
8. Wash the cells twice as step 4.
9. Suspend the pellet in 500 μL of FCM buffer. Analyze it immediately or suspend it in 0.5% paraformaldehyde/PBS and store the sample in a dark room at 2-8°C. Be sure to analyze it within 24 hours.

Consideration

A) If erythrocytes remain in the cell sample, we recommend hemolyzing them. If erythrocytes still remain after hemolysis, we recommend staining the cells with an anti-CD45 antibody and analyzing the results by lymphocyte gating.
B) We recommend using Clear Back (MBL, code no. MTG-001) to reduce the nonspecific staining of cells by endocytosis in macrophages.
C) For staining of *in vitro* cultured lymphocytes, we recommend staining with 7-AAD for exclusion of dead and non-viable cells.
D) Paraformaldehyde fixation of cells is not needed if the cells are analyzed within a couple of hours after staining.
E) If aggregation is found in the tube, please centrifuge it and use the supernatant to stain cells.
F) We strongly recommend Anti-PE or APC antibodies (e.g. MBL, code no. M240-3, M241-3) to enhance tetramer staining intensity (J. Immunol. 2015, 194:463-474).

Precautions

1. The reagent contains 0.09% of sodium azide. Sodium azide under acidic 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, where 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 should be disposed using the proper precautions.
3. Pipetting should never be done by mouth and contact of samples with skin and mucous membranes should be avoided.
4. Exposure of reagents to light should be minimized during storage or incubation.
5. The recommended cell survival rate of venous blood specimens is > 90%.
6. To obtain appropriate results with whole blood, we recommend keeping the test sample in a blood collection tube at room temperature and inverting the tube repeatedly just before staining. In order to obtain the appropriate result, do not use a cold test blood.
7. Do not incubate the cells with the hemolysis reagent for a long time, as prolonged incubation results in the disruption of leukocytes.
8. Erythrocytes of abnormal test blood, such as nucleated erythrocytes, and blood from patients with abnormal hemoglobin diseases, cannot be hemolyzed well. In such cases, the non-hemolyzed erythrocytes are incorrectly counted as leukocytes. This results in a falsely increased count of leukocytes and a falsely decreased count of MAIT cells.

Example of Staining:

Peripheral blood mononuclear cells (PBMCs) from healthy donors were collected from freshly isolated heparinized peripheral blood according to standard methods. The PBMCs were stained with T-Select Human MR1 Tetramer-PE loaded with 5-OP-RU or Ac-6-FP as the way described in the procedure.

The single lymphocyte population was defined using FSC/SSC and FSC-H/FSC-A gate and the viable cell population was defined using an FSC/7-AAD gate.

Numbers in the upper right quadrants represent the percentages of T-select Human MR1 Tetramer^{+} cells relative to the total CD3^{+} cells.