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T-Select MHC Tetramer

Mamu-A*90120-5 SIV gag Tetramer -SSVDEQIQW (50 tests)

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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 $CD8^+$ antigen-specific T cells requires cognate recognition of the T cell receptor (TCR) by a class I MHC/peptide complex. This can be done using T-Select MHC class I Tetramers which are composed of four MHC class I molecules each bound to the specific peptide 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 with a particular MHC molecule. Furthermore, since binding does not depend on functional pathways, this population includes specific $CD8^+$ 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. 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 monkey class I MHC Mamu-A*90120-5 and epitope peptide derived from SIV gag protein, and it can detect a Mamu-A*90120-5-restricted SIV gag-specific $CD8^+$ T cells.

The simian immunodeficiency virus (SIV) infects host $CD4^+$ T cells and proliferates, and destroys the host immune system to cause acquired immunodeficiency. SIV is considered to be the origin of the human immunodeficiency virus (HIV), which has recently become a worldwide epidemic, and has been used to investigate the mechanism of HIV infection and vaccine development strategies.

Matano T *et al.* (Institute of Medical Science, University of Tokyo) reported that the serum SIV load decreases significantly following SIV infection and vaccination with a Sendai virus vector expressing a CTL epitope in rhesus monkeys. They also revealed for the first time that the peptide at amino-acid positions 241–249 of the SIV gag is a CTL epitope restricted by Mamu-A*90120-5[1].

References for Mamu-A*90120-5 SIV $gag_{241\text{-}249}$

1) Tsukamoto T, *et al. J Virol* **83**: 9339-9346 (2009)
2) Ishii H, *et al. J Virol* **86**: 738-745 (2012)

MHC Restriction: Mamu-A*90120-5

Origin and Sequence of CTL Epitope

SIV gag (241-249 aa, SSVDEQIQW)

Conjugates

TS-M901-1: Streptavidin-Phycoerythrin (SA-PE)
Excites at 486-580 nm
Emits at 586-590 nm

TS-M901-2: Streptavidin-Allophycocyanin (SA-APC)
Excites at 633-635 nm
Emits at 660-680 nm

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.
The Monomer concentration is 100 µg/mL.

Storage Conditions

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

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).

Usage

This reagent is for use with standard flow cytometry methodologies.

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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.

Procedure for Peripheral Blood Mononuclear Cells

1. Prepare peripheral blood mononuclear cells (PBMC) according to established procedures. Cells should be re-suspended at a concentration of 2×10^7 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 PBMC into each test tube (e.g. 1×10^6 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 (15-25°C) protected from light.
7. Add any additional antibodies (e.g. anti-CD8) and vortex gently.
8. Incubate for 30 minutes at 2-8°C protected from light.
9. Add 3 mL of PBS or FCM buffer (2% FCS/0.05% NaN_3/PBS).
10. Centrifuge tubes at 400 x g for 5 minutes.
11. Aspirate or decant the supernatant.
12. Suspend the pellet in 500 µL of FCM buffer and 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.

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. 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.
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. It is reported that some anti-CD8 antibody clones may inhibit MHC tetramer from binding to the TCR.
D. A Tetramer that is constructed with the same allele of interest and an irrelevant peptide may be used as a negative control.
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.

References for T-Select MHC Tetramer

1) Altman JD, *et al. Science* **274**: 94-96 (1996)
2) Mcmichael AJ, *et al. J Exp Med* **187**: 1367-1371 (1998)
3) Bodinier M, *et al*. *Nat Med* **6**: 707-710 (2000)

Related Products

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

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