MHC Class II Human Tetramer – PE, APC, BV421

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

ALLELE AND PEPTIDE SPECIFICITY

MHC class II tetramers recognize human $CD4^+$ T cells that are specific for a particular peptide in combination with a class II HLA allele.

BACKGROUND

T lymphocytes play a central role in immune system function. Total T cell and T cell subset counts are measured by detection of various cell surface molecules. Detection of $CD4^+$ antigen-specific T cells requires cognate recognition of the T cell receptor (TCR) by a class II MHC/peptide complex. This can be done using MHC class II tetramers which are composed of a complex of four HLA MHC class II molecules each bound to the specific peptide[1,2] and conjugated with a fluorescent protein. Thus, MHC tetramer assays allow quantitation of the T cell population specific for a given peptide complexed in a particular MHC molecule. Furthermore, since binding does not depend on functional pathways, this population includes all specific $CD4^+$ T cells regardless of their functional status. Measurements may be performed in whole blood or isolated lymphocyte/mononuclear cell preparations or, in some cases where frequency is low, it may be necessary to perform an in vitro cell expansion[3]. Specific cell staining is accomplished by incubating the sample with the MHC tetramer reagent, then washing away excess tetramer. The number of tetramer positive lymphocytes is then determined by flow cytometry. MHC tetramer staining can be combined with other techniques, such as cell expansion and precursor frequency protocols[4,5].

REAGENTS

MHC class I tetramer: 50 tests, 500 µL

CONJUGATES

PE tetramers are labeled with Streptavidin-Phycoerythrin (SA-PE), excitation 486–580 nm/emission 586–590 nm.

APC tetramers are labeled with Streptavidin-Allophycocyanin (SA-APC), excitation 633–635 nm/emission 660–680 nm.

BV421 tetramers are labeled with Streptavidin-Brilliant Violet™ 421 (SA-BV421), excitation maximum 405 nm/emission maximum 421 nm.

The tetramer is dissolved in an aqueous buffer containing 0.5mM EDTA, 0.2% BSA, 0.01M Tris, 0.15M NaCl, and <0.1% NaN_3.

REAGENT PREPARATION

No preparation is necessary. MHC tetramer reagents are used directly from the vial after a brief vortex on low setting.

STORAGE CONDITIONS

Store at 2–8°C. Do not freeze. Minimize exposure to light.

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 (BV421 tetramer) to pink (PE tetramer) or light blue (APC tetramer) liquid.

USAGE

This reagent is for use with standard flow cytometry methodologies.

STATEMENT OF WARNINGS

1. This reagent contains <0.1% 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. Universal precautions should be observed whenever handling any potential infectious specimens or samples.
3. Never pipet 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.

MATERIALS REQUIRED BUT NOT SUPPLIED

- 12x75 mm polypropylene test tubes
- Transfer pipettes
- Pipettors and disposable pipette tips
- Vortex
- Centrifuge capable of 150 x g or 400 x g
- 37°C CO_2 incubator
- Aspirator
- PBS or FACS buffer (e.g. PBS with 0.2–1% BSA and 0.1% Sodium Azide)
- PBS with 0.5% paraformaldehyde or formalin
- RPMI-1640 supplemented with 2mM L-glutamine/5 x 10^{-5} M 2-Mercaptoethanol, 1mM sodium pyruvate, 1% Non-essential amino acids, 10mM HEPES, 100 µg/mL penicillin/streptomycin and 10% Human AB Serum
- Lyse Reagent (VersaLyse™ lysing solution, Beckman Coulter, Inc., PN A09777)
- Fixative Reagent (IOTest® 3 10x Fixative Solution, Beckman Coulter, Inc., PN A07800)
- Clear Back (MBL PN MTG-001)
- Human anti-CD4 antibody (Beckman Coulter clone SFCI12T4D11, PN 6602393, is recommended)

SYMBOL DEFINITIONS

= Store Away From Direct Light

= Expiration Date

= Number of Tests

= Amount

REF = Code Number

LOT = Lot Number

RUO = Research Use Only

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PROCEDURE FOR WHOLE BLOOD

1. Collect blood by venipuncture into a blood collection tube containing an appropriate anti-coagulant.
2. To each 12x75 mm test tube add 10 µL of MHC class II tetramer.
3. Add 200 µL of whole blood into each tube. Vortex gently.
4. Incubate for 2 hours 37°C in 5% CO_2 incubator‡.
5. Remove from incubator.
6. Add antibodies (e.g. anti-CD4), vortex gently, and incubate for 20 minutes at room temperature protected from light.
7. Lyse red blood cells using 2 mL of Lyse Reagent supplemented with 50 µL Fixative Reagent per tube.
8. Vortex for 5 seconds immediately after the addition of the Lyse/ Fixative solution.
9. Incubate for a minimum of 10 minutes at room temperature protected from light.
10. Centrifuge tubes at 150 x g for 5 minutes.
11. Aspirate or decant the supernatant.
12. Add 3 mL of PBS or FACS buffer.
13. Centrifuge tubes at 150 x g for 5 minutes.
14. Aspirate or decant the supernatant.
15. Resuspend the pellet in 500 µL of PBS with 0.1% formaldehyde. (12.5 µL Fixative Reagent/1 mL PBS).
16. Store prepared samples at 4°C protected from light for a minimum of 1 hour (maximum 24 hours) prior to analysis by flow cytometry.

PROCEDURE FOR PERIPHERAL BLOOD MONONUCLEAR CELLS

1. Prepare peripheral blood mononuclear cells (PBMC) according to established procedures. For staining, cells should be resuspended in RPMI complete media containing a suitable Fc receptor block, such as Clear Back (MTG-001), at a final concentration of 5 x 10^6 cells/mL§.
2. To each 12x75 mm test tube add 10 µL of MHC tetramer.
3. Add 200 µL (1 x 10^6) PMBC into each tube.
4. Vortex gently.
5. Incubate for 2 hours 37°C in 5% CO_2 incubator‡.
6. Remove from incubator.
7. Add antibodies (e.g. anti-CD4), vortex gently, and incubate for 20 minutes at room temperature, protected from light.
8. Add 3 mL PBS or FACS buffer.
9. Centrifuge at 400 x g for 5 minutes.
10. Aspirate the supernatant.
11. Resuspend the pellet in 500 µL of PBS with 0.5% formaldehyde. (62.5 µL Fixative Reagent/1 mL PBS).
12. Store prepared samples at 4°C protected from light for a minimum of 1 hour (maximum 24 hours) prior to analysis by flow cytometry.

‡Staining conditions may require optimization.

§ Enrichment procedures may be required to expand very rare subsets of antigen-specific CD4 T cells.

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 or frozen 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.
5. Although MHC tetramer reagents are held to strict quality control and purity standards, suitability for the end user's particular experimental system cannot be guaranteed.

SELECTED REFERENCES

1. Altman, J.D., Moss, P.H., Goulder, P.J., Barouch, D.H., McHeyzer, W., Bell, J.I., McMichael, A.J., and Davis. M.M.,1996. Phenotypic Analysis of Antigen-Specific T Lymphocytes. Science, 274:94-96.
2. McMichael, A.J., and O'Callaghan, C.A., 1998. A New Look at T Cells. J. Exp. Med., 187:1367-1371.
3. Nepom, G.T., Buckner, J.H., Novak, E.J., Reichstetter, S., Rejonen, H., Gebe, J., Wang, R., Swanson, E. and Kwok, W.W., 2002. HLA Class II Tetramers, Tools for Direct Analysis of Antigen-Specific $CD4^+$ T Cells. Arthritis and Rheumatism, 46: 5-12.
4. Novak, E.J., Nepom, G.T., Liu, A.W. and Kwok, W.W., 1999. MHC Class II Tetramers Identify Peptide-Specific Human $CD4^+$ T Cells Proliferation in Response to Influenza A Antigen. J. Clin. Invest., 104: R63-R67.
5. Lyons, A.B., and Doherty, K.V., 1998. Flow Cytometric Analysis of Cell Division by Dye Dilution. Current Protocols in Cytometry, 2:9.11.19.11.9. John Wiley and Sons, Inc.

TRADEMARKS

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EXAMPLE HISTOGRAMS

The following histograms are examples of whole blood and PBMC samples stained with MHC class II tetramer-PE and anti-CD4-ECD (clone SFCI12T4D11 "T4"). Region E, Panel A, represents the anitgen-specific $CD4^+$/MHC tetramer positive T cells.

Figure 1: Detection of Influenza HA-specific $CD4^+$ T cells in whole blood

Unstimulated whole blood samples were stained with anti-CD4-ECD and DRB1-01:01 HA tetramer (A) or DRB1-01:01 irrelevant tetramer (B).

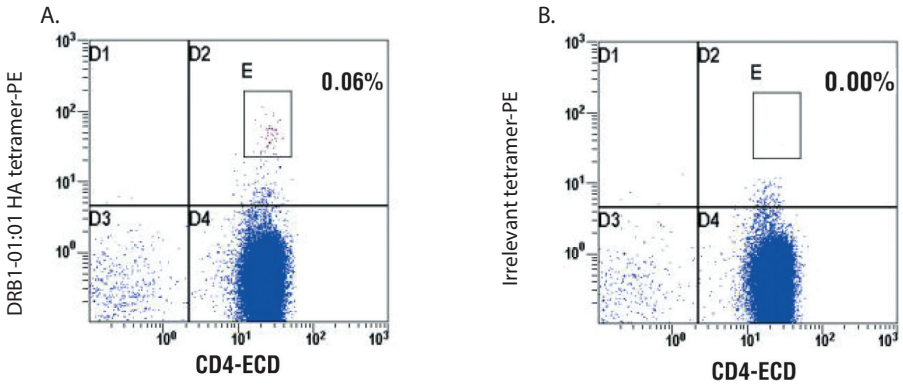

Figure 2: Expansion of Influenza HA-specific $CD4^+$ T cells in peptide-stimulated PBMCs

PBMCs were stimulated for 7 days with HA peptide and then stained with anti-CD4-ECD and DRB1-04:01 HA tetramer (A) or DRB1-04:01 irrelevant (gp39) tetramer (B).