MHC Class I Macaque Tetramer – PE, APC, BV421

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

ALLELE AND PEPTIDE SPECIFICITY

Macaque MHC class I tetramers recognize $CD8^+$ T cells that are specific for a particular peptide in combination with a class I Mamu (Rhesus Macaque) or Mafa (Mauritian Cynomolgus Macaque) MHC. The MHC molecule in this reagent has been modified to minimize CD8-mediated binding.[1]

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 $CD8^+$ 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 MHC class I tetramers[2], which are composed of a complex of four Mamu (Rhesus Macaque)[3–6] or Mafa (Mauritian Cynomolgus Macaque)[7,8] MHC class I molecules each bound to the specific peptide[2,3] and conjugated with a fluorescent-labeled Streptavidin. 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 $CD8^+$ T cells regardless of their functional status. Measurements may be performed in whole blood or isolated lymphocyte/mononuclear cell preparations. 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.

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
- Aspirator
- PBS or FACS buffer (e.g. PBS with 0.2–1% BSA and 0.1% Sodium Azide)
- PBS with 0.5% formaldehyde or equivalent commercial Fixative Reagent (e.g. IOTest® 3 10x Fixative Solution, Beckman Coulter, Inc., PN A07800)
- Commercial red blood cell Lyse Reagent (e.g VersaLyse™ lysing solution, Beckman Coulter, Inc., PN A09777 or equivalent)
- Clear Back (MBL PN MTG-001)
- Anti-CD8 antibody

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 tetramer and any additional antibodies (e.g. anti-CD8).‡
3. Add 200 µL of whole blood into each tube. Vortex gently.
4. Incubate for 30 minutes at room temperature protected from light.
5. Lyse red blood cells using 1 mL of Lyse Reagent supplemented with 0.2% formaldehyde Fixative Reagent per tube.
6. Vortex for 5 seconds immediately after the addition of the Lyse/ Fixative Solution per tube.
7. Incubate for a minimum of 10 minutes at room temperature protected from light.
8. Centrifuge tubes at 150 x g for 5 minutes.
9. Aspirate or decant the supernatant.
10. Add 3 mL of PBS or FACS buffer.
11. Centrifuge tubes at 150 x g for 5 minutes.
12. Aspirate or decant the supernatant.
13. Resuspend the pellet in 500 µL of PBS with 0.1% formaldehyde.
14. Store 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 buffer containing a suitable Fc receptor block, such as Clear Back (MTG-001), at a final concentration of $5x10^6$ cells/mL.
2. To each 12x75 mm test tube add 10 µL of MHC tetramer and any additional antibodies (e.g. anti-CD8).‡
3. Add 200 µL ($1x10^6$) PMBCs into each test tube.
4. Vortex gently.
5. Incubate for 30 minutes at room temperature protected from light.
6. Add 3 mL of PBS or FACS buffer.
7. Centrifuge tubes at 150 x g for 5 minutes.
8. Aspirate or decant the supernatant.
9. Resuspend the pellet in 500 µL of PBS with 0.5% formaldehyde.
10. Store 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.

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.
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. Bodinier, M., Peyrat, M-A., Tournay, C., Davodeau, F., Romagne, F., Bonneville, M., and Lang, F., 2000. Efficient Detection and Immunomagnetic Sorting of Specific T Cells Using Multimers of MHC Class I and Peptide with Reduced CD8 Binding. Nat. Med., 6:707-710
2. 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.
3. Kuroda, M.J., Schmitz, J.E., Barouch, D.H., Craiu, A., Allen, T.M., Sette, A., Watkins, D.I., Forman, M.A., and Letvin, N.L., 1998. Analysis of Gag-specific cytotoxic T lymphocytes in simian immunodeficiency virus-infected rhesus monkeys by cell staining with a tetrameric major histocompatibility complex class I-peptide complex. J. Exp. Med., 187:1373-1381.
4. Allen, T.M., Sidney, J., del Guercio, M., Glickman, R.L., Lensmeyer, G.L., Wiebe, D.A., DeMars, R., Pauza, C.D., Johnson, R.P., Sette, A., and Watkins, D.I., 1998. Characterization of the peptide binding motif of a rhesus MHC class I molecule (Mamu-A*01) that binds an immunodominant CTL epitope from simian immunodeficiency virus. J. Immunol., 160:6062-6071.
5. Reimann, K.A., Waite, B.C.D., Lee-Parritz, D.E., Lin, W., Uchanska-Ziegler, B., O'Connell, M.J., and Letvin, N.L., 1994. Use of human leukocyte-specific monoclonal antibodies for clinically immunophenotyping lymphocytes of rhesus monkeys. Cytometry, 17:102-108.
6. Allen, T.M., O'Connor, D.H., Jing, P., Dzuris, J.L., Mothé, B.R., Vogel, T.U., Dunphy, E., Liebl, M.E., Emerson, C., Wilson, N., Kunstman, K.J., Wang, X., Allison, D.B., Hughes, A.L., Desrosiers, R.C., Altman, J.D., Wolinsky, S.M., Sette, A. and Watkins, D.I., 2000. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. Nature, 407: 386-390.
7. Budde, M.L., Lhost, J.J., Burwitz, B.J., Becker, E.A., Burns, C.M., O'Connor, S.L., et al., 2011. Transcriptionally abundant major histocompatibility complex class I alleles are fundamental to nonhuman primate simian immunodeficiency virus-specific $CD8^+$ T cell responses. J. Virology, 85(7): 3250-3261.
8. Budde, M.L., Greene, J.M., Chin, E.N., Ericsen, A.J., Scarlotta, M., Cain, B.T., et al. 2012. Specific $CD8^+$ T cell responses correlate with control of simian immunodeficiency virus replication in Mauritian cynomolgus macaques. J. Virology, 86(14): 7596-7604.

TRADEMARKS

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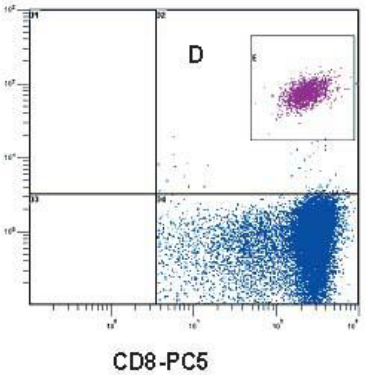
EXAMPLE HISTOGRAMS

Detection of gag-specific $CD8^+$ T cells in whole blood

Whole blood samples were stained with anti-CD8-PC5 (clone B9.11) and PE-labeled Mamu-A*01 SIV gag (CTPYDINQM) or Irrelevant tetramer. Gating strategy included a singlet gate based on FSC-H and FSC-A, followed by a $CD3^+$ gate and FSC x SSC gate. Region D represents the antigen-specific $CD8^+$ T cells detected by the tetramer.

Mamu-A*01 SIV gag tetramer-PE (T03004)



Irrelevant (Mamu-A*01 tat) tetramer-PE