

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

## ALLELE AND PEPTIDE SPECIFICITY

Human MHC class I tetramers recognize human CD8<sup>+</sup> T cells that are specific for a particular peptide in combination with a class I HLA allele. The HLA molecule in this reagent has been modified to minimize CD8-mediated binding.<sup>1</sup>

## 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<sup>+</sup> 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, which are composed of a complex of four HLA MHC class I molecules each bound to the specific peptide<sup>2,3</sup> 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<sup>+</sup> 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.

A human class I negative tetramer, with no known specificity, can be used in parallel to assess background fluorescence of PE (T01044), APC (T01054), or BV421 (T01044B), and allow for setting of regions/gates.

## 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<sub>3</sub>.

## 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)
- Human anti-CD8 antibody (Beckman Coulter clone SFC121Thy2D3, PN 6603861, is recommended)

## SYMBOL DEFINITIONS

-  = Store Away From Direct Light
-  = Expiration Date
-  = Number of Tests
-  = Amount
-  = Code Number
-  = Lot Number
-  = Research Use Only

#### 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).<sup>‡</sup>
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<sup>6</sup> cells/mL.
2. To each 12x75 mm test tube add 10 µL of MHC tetramer and any additional antibodies (e.g. anti-CD8).<sup>‡</sup>
3. Add 200 µL (1x10<sup>6</sup>) 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.

<sup>‡</sup> 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. McMichael, A.J., and O'Callaghan, C.A., 1998. A New Look at T Cells. *J. Exp. Med.*, 187:1367-1371.

#### TRADEMARKS

Brilliant Violet™ 421 is a trademark of Sirigen, and Sirigen is an entity of Becton Dickinson.

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MBL International is an exclusive licensee of MHC Tetramer technology.

US Patent Nos.: 5,635,363; 5,723,584; 5,874,239; 5,932,433 and 6,265,552. French Application No. FR 9911133.

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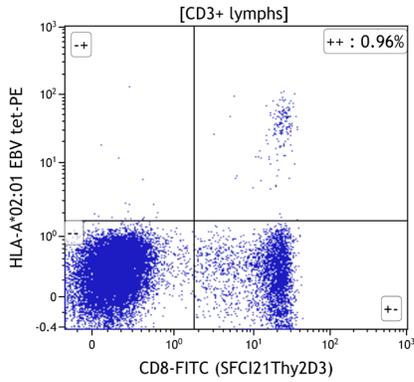
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EXAMPLE HISTOGRAMS

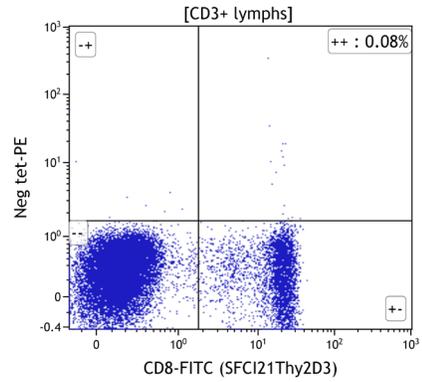
Detection of EBV-specific CD8<sup>+</sup> T cells in whole blood

Whole blood samples were stained with anti-CD3-PC7, anti-CD8-FITC (clone SFC121Thy2D3 "T8"), and HLA-A\*02:01 EBV (GLCTLVAML) or Negative tetramer. Gating strategy included a singlet gate based on FSC-H and FSC-A, followed by a CD3<sup>+</sup> gate and FSC x SSC gate. Percentages of CD8<sup>+</sup>/MHC tetramer positive T cells, derived from independent experiments, is shown.

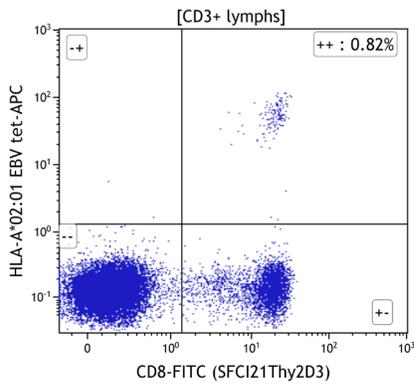
HLA-A\*02:01 EBV (GLCTLVAML) tetramer-PE (T01010)



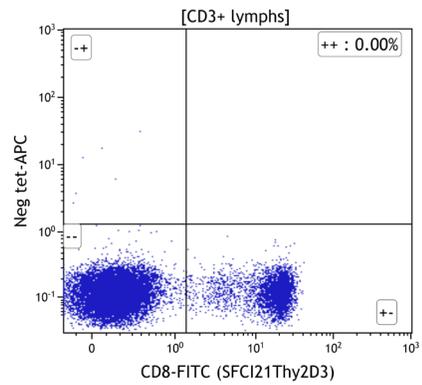
Negative tetramer-PE (T01044)



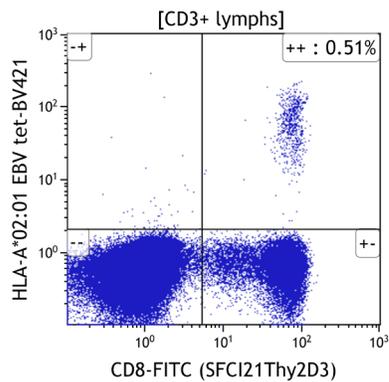
HLA-A\*02:01 EBV (GLCTLVAML) tetramer-APC (T01024)



Negative tetramer-APC (T01054)



HLA-A\*02:01 EBV (GLCTLVAML) tetramer-BV421 (T01010B)



Negative tetramer-BV421 (T01044B)

