

**MHC Tetramers and
Monomers for
Immuno-Oncology
and Autoimmunity
Drug Discovery**

MHC Tetramer Background

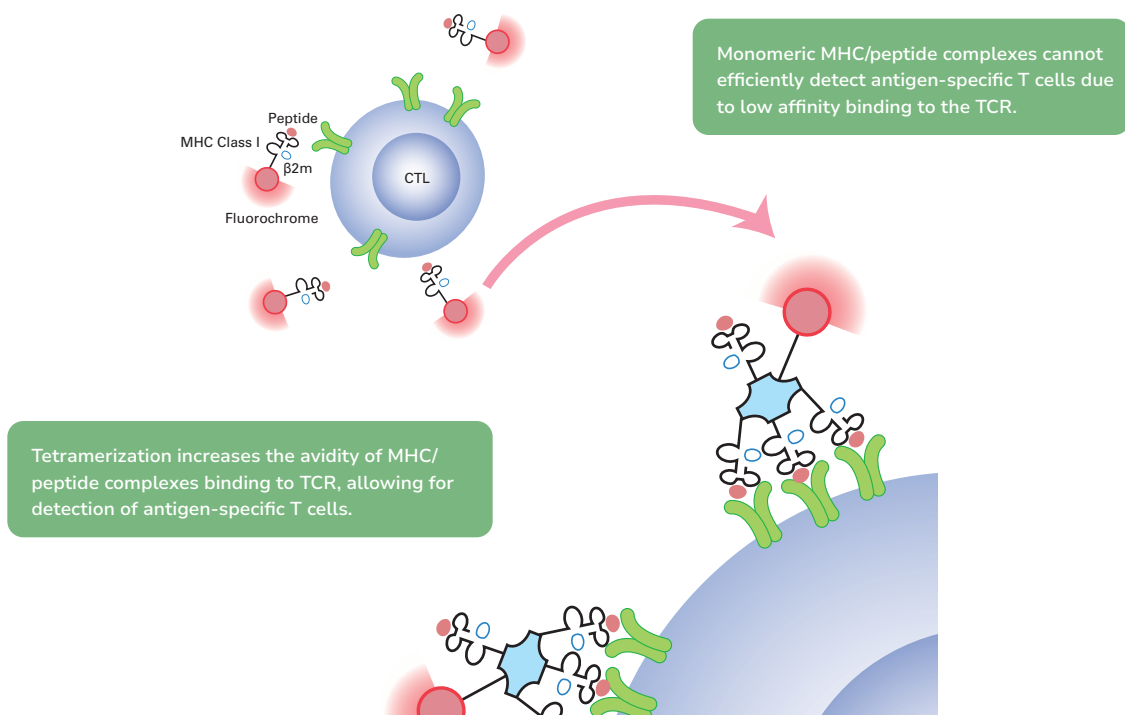
T-Cell Receptors recognize and bind to complexes composed of MHC molecules and specific peptides expressed on the surface of antigen-presenting cells. Biotinylation of MHC/peptide complexes followed by tetramerization with fluorochrome conjugated streptavidin increases their binding affinity. Therefore MHC tetramers bind stably to cognate T-cell receptors and consequently can be used as detection tools. Phycoerythrin (PE), allophycocyanin (APC), or Brilliant Violet™ 421 (BV421) fluorochromes are available for detection of antigen-specific T cells by flow cytometry or fluorescence microscopy using our MHC Multimers.

Class I and Class II MHC Tetramers

CD8+ T cells, also called cytotoxic T lymphocytes (CTLs), recognize complexes of MHC class I molecules and peptide. CTLs directly kill target cells including virus-infected and cancer cells. CD4+ T cells, which include helper and regulatory T cells, recognize complexes of MHC class II molecules and peptide. MHC Class I Tetramers are capable of detecting CD8+ T cells and MHC Class II Tetramers are capable of detecting CD4+ Class II Tetramers.

MHC Monomers

An MHC monomer is a sub-component of an MHC tetramer, made up of the MHC complex (α -chain plus β -2 microglobulin for class I or α -chain plus β -chain for class II) folded together with a specific peptide. Biotinylated monomers are available for most associated tetramer products. Biotinylated MHC monomers, the building blocks of MHC tetramers, are available for tetramer construction flexibility and novel applications.



Superior Quality and Specificity with MBLI's MHC Tetramers and Monomers

- Reduced Background for Human Alleles due to Patented $\alpha 3$ Mutation
- Exceptional Product Breadth: Class I and Class II, over sixty alleles, hundreds of unique targets
- Excellent Quality: Stringent QC to Ensure Product Performance

Patented $\alpha 3$ Mutation for Lower Background

Figure 1a

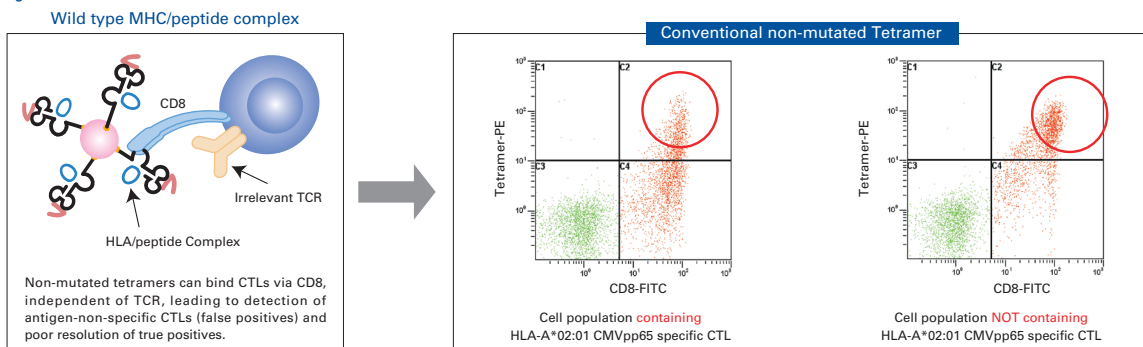
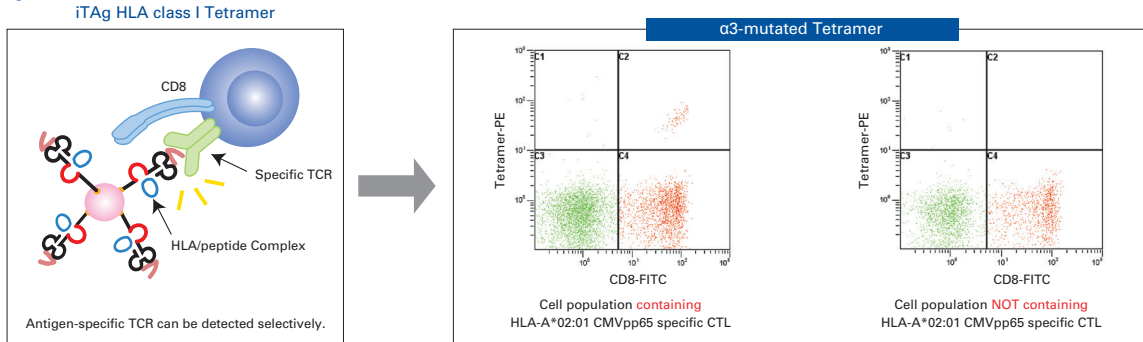


Figure 1b



The human leukocyte antigen (HLA) system is the name of the major histocompatibility complex in humans. CD8 molecules are known to assist binding of HLA to CTL in vivo and thus HLA molecules have binding sites for CD8 molecules. Bodinier et al. reported that introducing a mutation (A245V) in the HLA class I heavy chain $\alpha 3$ domain minimized unwanted binding to CD8 molecules and dramatically improved specificity (Nat. Med. 2000, 6: 707). MBLI has incorporated the patented mutation of the $\alpha 3$ domain in their HLA class I tetramers.

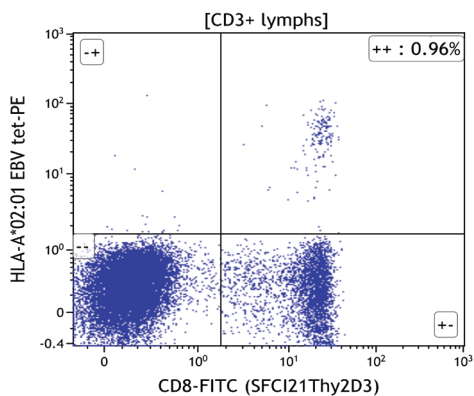
Common and Novel Tetramer Applications

- Detecting Antigen-Specific T Cells
- Immune Monitoring Assays
- Determining Vaccine Effectiveness
- Neoantigen Screening During Immunotherapy Drug Development
- *In vivo* studies (immunizing animal models)
- Characterization of T Cell Function
- Characterization of T Cell Receptors
- MHC Typing
- Peptide Library Screening
- Proliferation of Antigen-Specific T Cells
- Mass Cytometry (CyTOF)

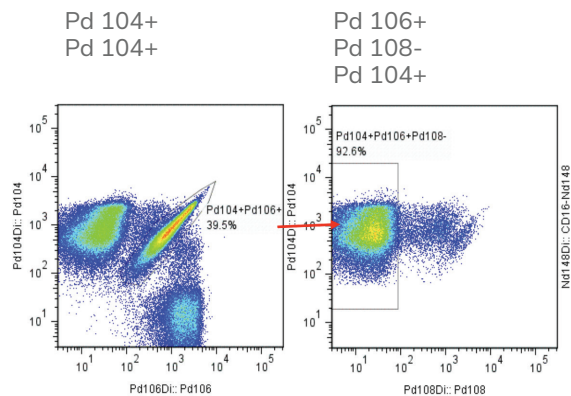
Emerging Utilities of Tetramers and Monomers

- Discovery and validation of neoepitopes
- Designing immune monitoring for vaccines and immunotherapeutics
- Isolation and expansion of peptide-specific T cells
- Vaccine design and development
- Assaying pre-existing anti-tumor responses in cancer patients
- Efficacy testing of immune checkpoint blockades and agonistic co-stimulatory biologics

Detection of EBV-specific T Cells in Blood



CyTOF using MHC Tetramers



MBLI is Committed to Meeting Your Unique Drug Discovery Needs

- Custom Tetramers for Novel Applications
- Custom Specificity Development
- Custom Allele Development
- Novel Immune Monitoring and Screening Solutions

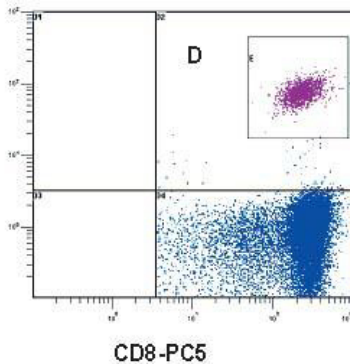
MBL has been developing and manufacturing MHC tetramer reagents in Japan since 2002. In 2013, we acquired an exclusive sublicense from Beckman Coulter to add the complete iTA^g™ MHC tetramer product line to the portfolio and constructed a state-of-the-art GMP facility for tetramer production in Des Plaines, IL. From the start of tetramer development, we have built an extensive knowledge base and gained valuable experience to provide high quality tetramer products and support to our customers.

Sample Data from Development Project Novell Allele Species (Rhesus Macaque)

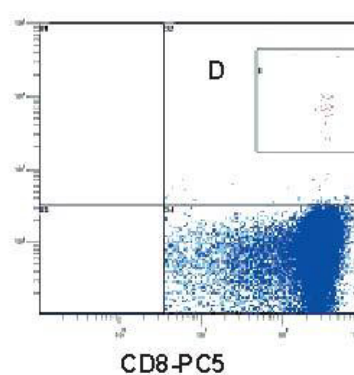
Detection of gag-specific CD8⁺ T cells in whole blood

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.

Whole Blood: CD8+/Mamu-gag+



Whole Blood: CD8+/Mamu-tat+

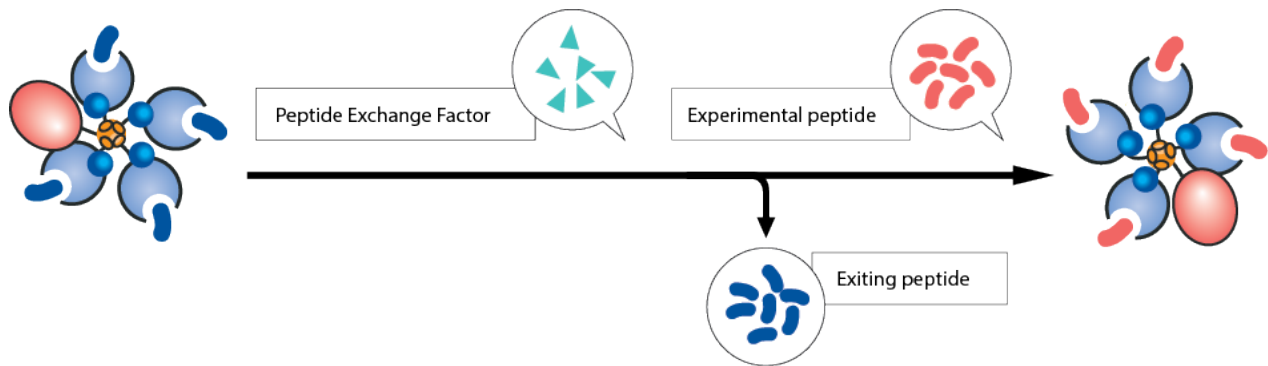


QuickSwitch™ Custom Tetramer Kits

High Throughput Immunogenic Peptide Discovery and Validation in 90 min

Functional screening of peptides for MHC class I binding is essential for vaccine design and immune monitoring. A rapid, high throughput and user friendly assay system that has the potential for clinical immune monitoring is needed now more than ever.

We present a 90-min-assay system that allows discrimination of MHC binding from non-binding peptides. This is particularly essential for the screening of immunogenic peptides from infectious agents or cancer neoantigens. Tetramers resulting from peptide exchange with selected peptides can then be used for immune monitoring.



QuickSwitch™ Platform Capabilities:

- Validate MHC binding peptides from in silico selected list of candidate peptides
- Generate new specificity tetramers for immune monitoring
- Perform functional stability studies for MHC binding peptides
- Compare epitopes to rank better binders and perform epitope mapping
- Quantitation of Peptide Exchange

Extensive Breadth of MHC Alleles Available

Related Products for Tetramer-Based Assays

Stock and Custom Peptides

MBL International's stock and custom peptides can be used for stimulation assays along with custom tetramer generation using the QuickSwitch™ custom tetramer kits. All peptides undergo a rigorous quality control process to ensure they are high grade including MS, LC-MS and HPLC analysis as well as COA documentation to ensure at least 95% purity.

A variety of sizes are available for custom peptides, from 1mg quantities up to 500mg and above. Peptide lengths can range from 2 amino acids to 100 amino acids depending on the peptide of interest.

Our stock peptides are specific to the MHC tetramer product line and contain peptides for popular disease targets such as CMV, MuLV, melanoma, prostate cancer and other virology and cancer targets. We offer over 150 stock peptides.

Flow Cytometry Reagents

MBL International offers over 2000 flow cytometry antibodies, isotype controls, RBC lysis buffers, permeabilization buffers and viability dyes. Popular checkpoint inhibitor products such as PD-1, PD-L1, CTLA-4, and TIM-3 antibodies are available. MBL International antibodies have a high signal to noise ratio with bright fluorochromes.

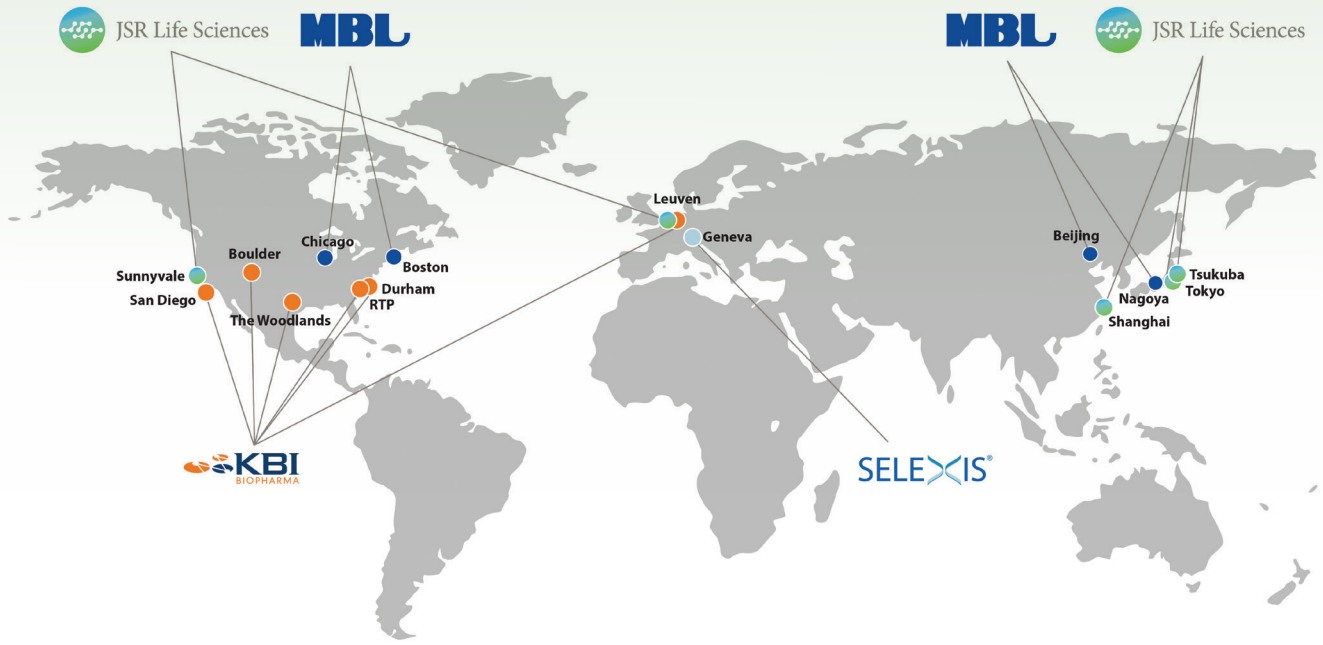
Available Alleles

MHC Tetramers				
Class I				
Human		Mouse	Rhesus Macaque	Chicken
HLA-A*01:01 HLA-A*02:01 HLA-A*02:06 HLA-A*02:07		H-2 K ^d	Mamu-A*01	BF2*1201
HLA-A*03:01 HLA-A*11:01 HLA-A*23:01 HLA-A*24:02		H-2 K ^b		BF2*1501
HLA-A*26:01 HLA-A*29:02 HLA-A*31:01 HLA-B*07:02		H-2 K ^k		
HLA-B*08:01 HLA-B*15:01 HLA-B*27:05 HLA-B*35:01		H-2 D ^d	Mauritian Cyno	Human-Mouse Chimeras
HLA-B*40:01 HLA-B*40:06 HLA-B*42:01 HLA-B*52:01		H-2 D ^b	Mafa-A1*063	A2K ^b
HLA-B*57:01 HLA-C*03:03 HLA-C*03:04 HLA-C*08:01		H-2 D ^k	Mafa-B*104:01	A24K ^b
HLA-C*12:02		H-2 L ^d		

Class II	
Human	Mouse Alleles
DPB1*04:01 DRB1*01:01 DRB1*03:01	I-Ab and I-Ad
DRB1*04:01 DRB1*04:05 DRB1*07:01	
DRB1*08:03 DRB1*09:01 DRB1*11:01	
DRB1*15:01 DRB1*15:02 DRB4*01:01	

Other Tetramers
CD1d: Human and Mouse
Human Non-Classical Class I: HLA-E*01:01 and HLA-E*01:03
Mouse Non-Classical Class I: Qa-1b
New alleles are always in development. If you do not see your allele of interest, please contact us.

Visit MBLINTL.com for the Latest Alleles Available!



MBL International: A Leading Provider for Immuno-Oncology Drug Discovery

MBL International

MBL International Corporation (MBLI) is a leading life science company focused on providing high quality, innovative, solutions-based products for both life science research and clinical diagnostics. Our products are used widely in academic research institutions, pharmaceutical and biotechnology companies, government agencies as well as hospital and reference laboratories.

MBLI develops, manufactures, and markets a broad portfolio of monoclonal and polyclonal antibodies, MHC tetramers and ELISA kits for research related to allergy, apoptosis, autophagy, cancer, epigenetics, immunology, and neuroscience. We have recently added new products including flow cytometry, exosome research products, and 3D cell culture products to our portfolio.

JSR Life Sciences

MBL International is a division of JSR Life Sciences and a business unit of JSR Corporation.

JSR Life Sciences continues to develop highly functional materials and products to meet current and future needs of the growing biotechnology industry. JSR Life Sciences provides materials that contribute to the manufacturing process of biopharmaceuticals, life science research applications, in vitro diagnostics and medical devices.

JSR Corporation is an international company employing over 6,000 people worldwide and being a leading materials supplier in a variety of technology driven markets. JSR Corporation's global network is headquartered in Tokyo (Japan) and has factories and offices all over the world.