

Flex-T™ HLA-A*03:01 Monomer UVX

Catalog# / Size	280005 / 50 µg
Regulatory Status	RUO
Other Names	MHC tetramer, HLA tetramer, HLA monomer, MHC monomer, biotinylated HLA monomer

Description CD8⁺ T cells are essential components of the immune system. They function through TCR recognition of antigens (peptide epitopes) presented by the class I MHC (HLA in humans) molecules. Thus, TCR recognition of cognate MHC/peptide complex can be utilized for the detection of antigen-specific CD8⁺ T cells. Due to the intrinsic low affinity of MHC/TCR interaction, the detection of antigen specific T cells requires enhanced binding avidity by multimerization of MHC/peptide monomers, ensuring the interaction of multiple MHC/TCR simultaneously. This can be accomplished by using streptavidin to tetramerize biotinylated MHC class I complexes.

In the absence of proper peptide binding, the HLA class I complex will quickly disassociate. Flex-T™ UVX (Ultraviolet Exchange) monomer is designed as a biotinylated α-chain, associated with the β2-microglobulin chain, and stabilized by a UV-labile peptide. The C-terminus of the α-chain is site-specifically biotinylated by the enzyme BirA. When the complex (monomer) is irradiated with UV in the presence of a new peptide with proper affinity, it will result in a net peptide exchange. The new peptide replaces the pre-loaded UV-labile peptide and forms a new MHC monomer. The new monomers can be then tetramerized and used for labeling antigen-specific T cells or other studies.

Product Details

Verified Reactivity	Human
Formulation	PBS (pH 7.5) containing 0.5% BSA and protease inhibitors.
Concentration	0.2 mg/ml
Storage & Handling	Store frozen (-20 or -80°C); shipped in blue ice. Aliquot upon receipt before freezing; avoid repeated freeze-thaw cycles. Protect from light.
Application	ELISA - Quality tested FC - Verified
Recommended Usage	For flow cytometric staining, the suggested use of this reagent is 20µl of 0.2mg/ml biotinylated monomer, mixed with 20µl of 400µM peptide, following our recommended protocol. This will generate tetramers sufficient for 15 tests (single color staining) or 7 tests (two-color staining).
Application Notes	<p>Flex-T™ HLA-A*03:01 Monomer UVX offers the flexibility of exchanging the pre-loaded UV-sensitive peptide to other antigenic peptides of interest before assembling tetramers for flow cytometry analysis. This product can also be used for the screening of peptide epitopes that are able to form a stable complex with the HLA-A*03:01 allele.</p> <p>Two-color staining with the same allele/peptide combination is recommended for flow cytometry analysis to increase the resolution and specificity of the assay.</p> <p>If peptides already characterized and published are preferred, click here to view.</p> <p>To order custom products please fill out the Custom Flex-T™ Request Form.</p>

Application References

(PubMed link indicates BioLegend citation)

1. Altman JD, *et al.* 1996. *Science* 274:94-6.
2. Rodenko B, *et al.* 2006. *Nat. Protoc.* 1:1120.
3. Toebe M, *et al.* 2006. *Nat. Med.* 12:246.
4. Bakker AH, *et al.* 2008. *Proc. Natl. Acad. Sci. USA* 105:3825.
5. Bieling, M. *et al.* 2018. *Oncotarget*. 9(4):4737-57. (ELISA) [PubMed](#)

Product Citations

1. Bieling M, *et al.* 2018. *Oncotarget*. 9(4):4737-57. [PubMed](#)

Antigen Details

Biology Area	Immunology
Molecular Family	MHC Antigens
Gene ID	NA

Related Protocols

[Intracellular Cytokine Staining Protocol - Video](#)

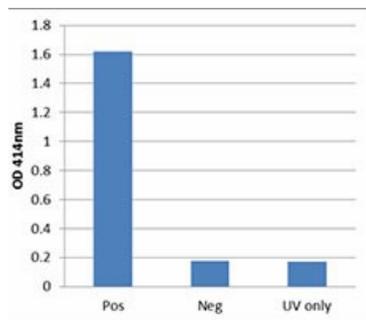
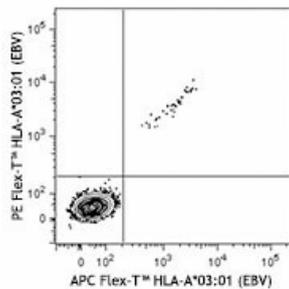
[Flex-T™ HLA Class I ELISA Protocol](#)

[Cell Surface Flow Cytometry Staining Protocol](#)

[Intracellular Flow Cytometry Staining Protocol](#)

[Flex-T™ Tetramer Preparation and Flow Cytometry Staining Protocol](#)

Product Data



Flex-T™ HLA-A*03:01 monomer UVX was subjected to UV-activated peptide exchange with an EBV peptide (RLRAEAQVK). The resulting new monomer was assembled into tetramers with PE- or APC-conjugated streptavidin. Human PBMCs from an HLA-A3 positive donor were stained with both PE- and APC-labeled tetramers. Data shown were cells gated on live CD8⁺ (CD4, CD19, CD14, CD16)⁻ lymphocytes.

The bar graph shows ELISA results after peptide exchange on Flex-T™ HLA-A*03:01 monomer UVX. The UVX monomer was irradiated with UV light in the presence of a positive (Pos) or negative (Neg) peptide, or no peptide (UV only). The positive peptide is an HLA-A3 epitope from melanosomal protein gp100 (LIYRRRLMK); the negative peptide is an HLA-B7 epitope from RSV (NPKASLLSL).

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