Flex-T™ Tetramer and Cell Staining Protocol V.3

Sam Li¹

¹BioLegend

Sam Li
BioLegend

ABSTRACT

Using UV-induced peptide exchange, MHC/peptide monomers can be generated with conditional Flex-T™ monomers that harbor peptides of interest in their binding grooves. These new MHC monomers are subsequently multimerized using streptavidin-fluorophore conjugates. The resulting Flex-T™ reagents can be used for staining antigen-specific T cells and flow cytometric analysis. In humans, the MHC molecules are called HLA (Human Leukocyte Antigen).

GUIDELINES

Representative data:

DOI:
[dx.doi.org/10.17504/protocols.io.babeiaje](https://doi.org/10.17504/protocols.io.babeiaje)

External link:
<https://www.biolegend.com/protocols/flex-t-tetramer-preparation-and-flow-cytometry-staining-protocol/4251/>

Protocol Citation: Sam Li . Flex-T™ Tetramer and Cell Staining Protocol.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.babeiaje>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Created: Dec 09, 2019

Last Modified: Dec 09, 2019

PROTOCOL integer ID:
30790

Keywords: tetramer, flex-t, cell staining, flow cytometry

A) CD8⁺T Cells previously gated on lymphocytes (FSC vs SSC) and 7-AAD negative events, were stained with FITC anti-CD8a and an exclusion cocktail containing Alexa Fluor® 700 anti-CD4, CD19, CD14, and CD16.

B) Antigen specific CD8⁺T Cells, gated as described, were detected with Flex-T™ tagged with PE and APC. HLA-A*11:01 Flex-T™ was loaded with an EBV peptide (IVTDFSVIK).

Chart Protocol:

Fluorescent tetramer generation and cell staining diagram

MATERIALS

MATERIALS

☒ Phosphate Buffered Saline (PBS) **BioLegend Catalog #926201**

☒ Cell Staining Buffer **BioLegend Catalog #420201**

☒ Plate Sealers **BioLegend Catalog #423601**

☒ Peptide Flex-T™ monomer UVX **BioLegend**

Reagents:

- DMSO (e.g. Sigma-Aldrich Cat#D5879)
- 50mM D-Biotin (e.g. Thermo Fisher, Cat#B20656)
- 10% (w/v) NaN₃ (e.g. Sigma, Cat#S2002)
- Fluorophore-conjugated Streptavidin (BioLegend Cat. [405203](#), Cat. [405207](#), Cat. [405225](#) or equivalent)
- 96-well Polypropylene Microplate, V-shape (e.g. Greiner bio-one cat. # 651201)
- 96-well Polystyrene Microplate, U-shape (e.g. Falcon Cat#353077) or 5mL, 12 x 75mm tubes (e.g. Falcon Cat# 352008)
- 1.5ml tubes (e.g. Eppendorf Cat# 022364111)
- For proteogenomic applications, compatible with our TotalSeq™ product line, use one of our oligo [barcoded fluorophore-conjugated](#) Streptavidin reagents

Equipment:

- UV lamp, long-wave UV, 366 nm, 8 Watts (For example CAMAG cat# 022.9115, or Ultraviolet Crosslinker CL-1000L)
- Incubator (37°C)
- Centrifuge capable of accommodating microtiter plates and tubes
- Single and multichannel pipettes capable of accurate delivery of variable volumes, and pipette tips

Tips:

- DMSO can be used to dissolve the peptides. However, do not exceed an end concentration of 10% (v/v) in the exchange reaction.
- Avoid repeated freeze-thawing.
- The Flex-T™/peptide solution needs to be kept on ice in the dark as much as possible. Do not work in front of a window.
- The use of short-wavelength (254 nm) or broad-band UV lamps is detrimental to MHC complexes.
- Centrifuge all vials before use (1 minute 2500 x g at 4°C).

Peptide Exchange:

- 1 Bring all reagents to 0°C by putting them on ice.

- 2 Dilute 10mM stock solutions of peptides of choice to 400μM by mixing 5μl of peptide stock solution with 120μl PBS, and keep on ice.
- 3 Add 20μl diluted peptide (400μM) and 20μl peptide Flex-T™ monomer UVX (200μg/ml) into 96-well V bottom plate. Mix by pipetting up and down.
- 4 Seal the plate; centrifuge at 2500xg for 2 minutes at 4°C to collect the liquid down
- 5 Remove the seal; put the plate on ice and illuminate with UV light for 30 minutes (the distance of the UV lamp to the samples should be 2-5cm).
- 6 Seal the plate; incubate for 30 minutes at 37°C in the dark.
- 7 To evaluate the efficiency of the peptide exchange follow the Protocol for HLA class I ELISA to evaluate peptide exchange.

Generation of Tetramers:

- 8 Transfer 30μl of peptide-exchanged monomer into a 1.5ml Eppendorf tube, or a new plate, then add 3.3μl of conjugated streptavidin, mix by pipetting up-and-down. Incubate on ice in the dark for 30 minutes. This is enough for about 15 tests. Note: BioLegend fluorophore-conjugated streptavidin products are recommended. For 30μl of exchanged Flex-T™ monomer we suggest using 3.3μl of BioLegend PE-streptavidin (Cat#[405203](#)) or APC- streptavidin (Cat#[405207](#)). For BV421-streptavidin conjugate (Cat#[405225](#)) use 1.3 μl. For oligo [barcoded](#) Streptavidin reagents please use 1.3 μl. For optimal reaction with other fluorophore-conjugated streptavidin products ensure that the monomer:streptavidin conjugate has a 5:1 ~ 6:1 molar ratio. (For our full choice of Streptavidin conjugates, visit: www.biolegend.com/streptavidin_conjugates. Note that purified, biotinylated, HRP, MojoSort™, and Ultra Streptavidin (USA) kits are not recommended for this

procedure.)

- 9 During the incubation, prepare blocking solution by adding 1.6µl 50mM D-Biotin and 6µl 10% (w/v) NaN₃ to 192.4µl PBS, mix by vortexing. After the incubation, add 2.4µl of blocking solution and pipette up-and-down to stop the reaction.
- 10 Incubate the tubes or sealed plates at 2-8°C overnight (or on ice for 30 minutes in the dark, if staining needs to be performed immediately). Tip: We recommend Flex-T™ to be assembled with two different streptavidin conjugates in separate reactions. This allows for two-color staining with the same tetramer allele, ensuring the highest specificity.

Cell Staining and Flow Cytometric Analysis:

- 11 Prepare cells of interest
- 12 Prior to performing staining, centrifuge the assembled tetramers in tube or plate at 2500xg for 5 minutes at 4°C. Then keep on ice in the dark.
- 13 Add 2 x 10⁶ cells to 12 x 75mm tubes or a 96-well U-bottom plate. Adjust volume to 200µl with Cell Staining Buffer. Add 2µl per sample of Flex-T™ complex prepared in Steps 7-9, mix and incubate on ice in the dark for 30 minutes.
- 14 If co-staining with surface antibodies, prepare the antibody cocktail based on optimal staining concentration of each reagent. Incubate for 30 minutes on ice in the dark.
- 15 Wash the cells with Staining Buffer two times. Resuspend cells with Staining Buffer.
- 16 Acquire the samples with a flow cytometer and appropriate settings within 2 hours. Tip: A titration of the Flex-T™ is recommended for optimal performance.