# Flex-T™ Tetramer and Cell Staining Protocol

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## 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).

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## Guidelines

### Materials

- Phosphate buffered saline pH 7.4, 10X concentrate (PBS, BioLegend Cat# 926201)
- Conditional Flex-T™ monomers
- 10 mM peptide solution of choice in 100% DMSO
- DMSO (e.g. Sigma-Aldrich Cat#D5879)
- 50 mM 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)
- Cell Staining Buffer (BioLegend Cat#420201 or equivalent)
- 96-well Polystyrene Microplate, U-shape (e.g. Falcon Cat#353077) or 5mL, 12 x 75mm tubes (e.g. Falcon Cat# 352008)
- Plate sealers (BioLegend Cat# 423601)
- 1.5 mL tubes (e.g. Eppendorf Cat# 022364111)

### Equipment

- UV lamp, long-wave UV, 366 nm, 8 Watts (For example CAMAG cat# 022.9115, or Ultraviolet Crosslinker CL-1000)
- 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

### Precautions for use

- 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 3000xg at 4°C).

**View protocol on Biolegend website for Representative Data**

## Protocol

Peptide exchange

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

Peptide exchange

**Step 2.**
Dilute 10mM stock solutions of peptides of choice to 400µM by mixing 5µl of peptide stock solution with120µl PBS, and keep on ice.

Peptide exchange

**Step 3.**

Add 20µl diluted peptide (400µM) and 20µl conditional Flex-T™ monomer (200µg/mL) into 96-well U-bottom plate. Mix by pipetting up and down.

Peptide exchange

**Step 4.**
Seal the plate; centrifuge at 3300xg for 2 minutes at 4°C to collect the liquid down.

Peptide exchange

**Step 5.**
Remove the seal; put the plate on ice and illuminate with UV light for 30 minutes (the distance of the UVlamp to the samples should be 2-5 cm).

Peptide exchange

**Step 6.**
Seal the plate; incubate for 30 minutes at 37°C in the dark.

Peptide exchange

**Step 7.**
To evaluate the efficiency of the peptide exchange follow the Protocol for HLA class I ELISA to evaluatepeptide exchange.

Generation of Tetramers

**Step 8.**

Transfer 30µl of peptide-exchanged monomer into a new plate, then add 3.3µl of conjugatedstreptavidin, mix by pipetting up-and-down. Incubate on ice in the dark for 30 minutes. This is enoughfor about 15 tests.

*Note: BioLegend fluorophore-conjugated streptavidin products are recommended. For 30µl ofexchanged Flex-T™ monomer we suggest to use 3.3µl of BioLegend PE-streptavidin (Cat#405203) orAPC- streptavidin (Cat#405207). For BV421-streptavidin conjugate (Cat#405225) use 1.3µl. Foroptimal reaction with other fluorophore-conjugated streptavidin products ensure that themonomer:streptavidin conjugate has a 6:1 molar ratio.*

## Generation of Tetramers

**Step 9.**

During the incubation, prepare blocking solution by adding 1.6µl 50mM D-Biotin and 6µl 10% (w/v) NaN3to 192.4µl PBS, mix by vortexing. After the incubation, add 2.4µl of blocking solution and pipette upand-down to stop the reaction.

## Generation of Tetramers

**Step 10.**

Seal the plate and incubate at 2 - 8°C overnight (or on ice for 30 minutes in the dark).

*Note: We recommend Flex-T™ to be assembled with two different streptavidin conjugates inseparate reactions. This allows for two-color staining with the same tetramer allele, ensuring thehighest specificity.*

## Cell staining and flow cytometric analysis

**Step 11.**

Prepare cells of interest

## Cell staining and flow cytometric analysis

**Step 12.**

Prior to perform staining, centrifuge the plate at 3300xg for 5 minutes at 4°C. Keep on ice in the dark.

## Cell staining and flow cytometric analysis

**Step 13.**

Add 2 x $10^6$ cells to a 96-well U-bottom plate or 12 x 75 mm tubes. Adjust volume to 200µl with CellStaining Buffer. Add 2µl per sample of Flex-T™ complex prepared in Steps 7 - 9, mix and incubate on icein the dark for 30 minutes.

## Cell staining and flow cytometric analysis

**Step 14.**

If co-staining with surface antibodies, prepare the antibody cocktail based on optimal stainingconcentration of each reagent. Incubate for 30 minutes on ice in the dark.

## Cell staining and flow cytometric analysis

**Step 15.**

Wash the cells with Staining Buffer two times. Resuspend cells with Staining Buffer.

## Cell staining and flow cytometric analysis

**Step 16.**

Acquire the samples with a flow cytometer and appropriate settings within 2 hours.

*Note: A titration of the Flex-T™ is recommended for optimal performance.*