Flex-T™ Tetramer and Cell Staining Protocol V.1

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http://www.biolegend.com/media_assets/support_protocol/Protocol%20for%20fluorescent%20tetramer%20generation%20and%20cell%20staining%2006202016.pdf

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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).

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 × 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

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 with120µl PBS, and keep on ice.

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.

4 Seal the plate; centrifuge at 3300xg 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 UVlamp to the samples should be 2-5 cm).

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 evaluatepeptide exchange.

Generation of Tetramers

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.

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.

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

11 Prepare cells of interest

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

13 Add 2 × 10^6 cells to a 96-well U-bottom plate or 12 × 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.

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.

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.

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