Flex-T™ Tetramer and Cell Staining Protocol Version 2

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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 with 120µl 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.

DURATION
00:02:00

Peptide exchange

Step 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 2-5 cm).

DURATION
00:30:00

Peptide exchange

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

DURATION
00:30:00

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 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 exchanged 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. For optimal reaction with other fluorophore-conjugated streptavidin products ensure that the monomer: streptavidin conjugate has a 6:1 molar ratio.

DURATION
00:30:00

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 up-and-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 in separate reactions. This allows for two-color staining with the same tetramer allele, ensuring the highest specificity.

DURATION
00:30:00

Cell staining and flow cytometric analysis

Step 11.
Prepare cells of interest

Cell staining and flow cytometric analysis

Step 12.

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

DURATION

00:05:00

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 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.

DURATION

00:30:00

Cell staining and flow cytometric analysis

Step 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.

DURATION

00:30:00

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.