Jun 29, 2020

Flex-T™ Fixed Peptide Tetramer Preparation and Flow Cytometry Staining Protocol

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This protocol is optimized to generate MHC tetramers using our streptavidin fluorophore conjugates and fixed peptide monomers. Unlike our UV-exchangeable monomers (denoted as UVX), the fixed peptide monomers are ready to use and do not need a peptide exchange. The resulting Flex-T™ tetramers can be used for staining antigen-specific T cells and flow cytometric analysis. In humans, the MHC molecules are called HLA (Human Leukocyte Antigen).

EXTERNAL LINK

https://www.biolegend.com/en-us/protocols/flex-t-fixed-peptide-tetramer-preparation-and-flow-cytometry-staining-protocol

PROTOCOL CITATION

Sam Li 2020. Flex-T™ Fixed Peptide Tetramer Preparation and Flow Cytometry Staining Protocol. **protocols.io** https://protocols.io/view/flex-t-fixed-peptide-tetramer-preparation-and-flow-bhvtj66n

EXTERNAL LINK

https://www.biolegend.com/en-us/protocols/flex-t-fixed-peptide-tetramer-preparation-and-flow-cytometry-staining-protocol

CREATED

Jun 24, 2020

LAST MODIFIED

Jun 29, 2020

PROTOCOL INTEGER ID

38547

GUIDELINES

Tips:

- Avoid repeated freeze-thawing.
- Avoid exposure to light as much as possible when performing this protocol. Do not work in front of a window.
- Centrifuge all vials before use (1 minute 2500 x *g* at 4°C).

MATERIALS

NAME	CATALOG #	VENDOR
Phosphate Buffered Saline (PBS)	926201	BioLegend
Cell Staining Buffer	420201	BioLegend

NAME	CATALOG #	VENDOR
PE Streptavidin	405203	BioLegend
APC Streptavidin	405207	BioLegend
Brilliant Violet 421™ Streptavidin	405226	BioLegend

MATERIALS TEXT

Reagents

- Phosphate buffered saline pH 7.4, 10X concentrate (PBS, BioLegend Cat. No. 926201)
- Fixed peptide Flex-T™ biotin monomer
- 50 mM D-Biotin (e.g. Thermo Fisher, Cat. No. B20656)
- 10% (w/v) NaN_3 (e.g. Sigma, Cat. No. S2002)
- Fluorophore-conjugated Streptavidin (BioLegend Cat. No. 405203, 405207, 405225 or equivalent)
- Cell Staining Buffer (BioLegend Cat. No. 420201 or equivalent)
- 1.5 mL tubes (e.g. Eppendorf Cat. No. 022364111)
- For proteogenomic applications compatible with our TotalSeq™ product line, use one of our oligo barcoded fluorophore-conjugated Streptavidin reagents.

Equipment

- Centrifuge capable of accommodating microtiter plates and tubes
- Single and multichannel pipettes capable of accurate delivery of variable volumes
- Pipette tips

Generation of Tetramers:

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

2 Transfer 30 µL of fixed peptide monomer into a 1.5 mL Eppendorf tube, or a new plate, then add 3.3 µL of conjugated streptavidin and 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 30 µL of fixed peptide Flex-T™ monomer, we suggest using 3.3 µL of BioLegend PE Streptavidin (Cat. No. 405203) or APC Streptavidin (Cat. No. 405207). For BV421™ Streptavidin (Cat. No. 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 our Streptavidin Conjugates webpage. Note that purified, biotinylated, HRP-Streptavidin and MojoSort™ and Ultra Streptavidin (USA) kits are not recommended for this procedure.)

3 During the incubation, prepare blocking solution by adding 1.6 µL 50 mM D-Biotin and 6 µL 10% (w/v) $NaN_3$to 192.4 µL PBS and mix by vortexing. After the incubation, add 2.4 µL of blocking solution and pipette up and down to stop the reaction.

4 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:

5 Prepare cells of interest.

6 Prior to performing staining, centrifuge the assembled tetramers in tubes or a plate at 2500 x*g*for 5 minutes at 4°C. Then keep on ice in the dark.

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

8 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.

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

10 Acquire the samples on a flow cytometer with appropriate settings within 2 hours.
Tip: A titration of the Flex-T™ is recommended for optimal performance.