QuickSwitch™ Tetramer Kit

- QuickSwitch™ HLA-A*02:01 Tetramer Kit-PE (Code No: TB-7301-K1)
- QuickSwitch™ HLA-A*02:01 Tetramer Kit-APC (Code No: TB-7301-K2)
- QuickSwitch™ HLA-A*02:01 Tetramer Kit-BV421 (Code No: TB-7301-K4)

For Research Use Only. Not for use in diagnostic procedures.

APPLICATION

The QuickSwitch™ Tetramer Kit utilizes a patented technique for exchanging up to ten peptides on an MHC class I tetramer. New specificity tetramers obtained by peptide exchange can then be used for identification of antigen-specific CD8+ T lymphocytes in staining assays.

SUMMARY AND EXPLANATION

Major histocompatibility complex (MHC)-encoded glycoproteins bind peptide antigens through non-covalent interactions to generate complexes that are displayed on the surface of antigen-presenting cells for recognition by T cells. Peptide-binding site occupancy is necessary for stable assembly of newly synthesized MHC proteins and export from the endoplasmic reticulum. During this stage peptides produced in the cytosol compete for binding to MHC molecules, resulting in extensive peptide exchanges that are regulated by accessory molecules, such as tapasin.[1,2] The QuickSwitch™ Tetramer Kit is based on the capacity of MHC class I molecules to exchange peptides

PRINCIPLE

The kit contains an MHC class I tetramer made from monomer units folded with an irrelevant exchangeable peptide, along with a proprietary Peptide Exchange Factor, for the generation of tetramers loaded with specific peptides of interest.

KIT COMPONENTS

QuickSwitch™ Tetramer

MHC class I tetramer, whose monomer content is 50 µg/mL, in a buffered solution with added protein stabilizers and ≤0.09 % sodium azide (500 µL x 1 amber vial with amber cap). Keep away from direct light. Store at 2-8°C.

Peptide Exchange Factor

The proprietary Peptide Exchange Factor contains ≤0.09 % sodium azide (13 µL x 1 clear vial with green cap). Store at ≤ -20°C.

CONJUGATES

PE tetramers are labeled with Streptavidin-Phycoerythrin (SA-PE), excitation 486–580 nm/emission 586–590 nm.

APC tetramers are labeled with Streptavidin-Allophycocyanin (SA-APC), excitation 633–635 nm/emission 660–680 nm.

BV421 tetramers are labeled with Streptavidin-Brilliant Violet™ 421 (SA-BV421), excitation maximum 405 nm/emission maximum 421 nm.

STORAGE CONDITIONS

The Peptide Exchange Factor must be frozen at ≤-20°C upon kit arrival, and the QuickSwitch™ Tetramer is stored at 2-8°C.

EVIDENCE OF DETERIORATION

Any change in the physical appearance of this reagent may indicate deterioration, and the reagent should not be used. The normal appearance is a clear, colorless (BV421 tetramer) to pink (PE tetramer) or light blue (APC tetramer) liquid.

WARNINGS AND PRECAUTIONS

1. QuickSwitch™ Tetramer is light sensitive and therefore should be protected from light during storage and during all the steps of the assay.
2. Avoid microbial contamination of all reagents involved in the testing procedure or incorrect results may occur.
3. Incubation times or temperatures other than those specified may give erroneous results.
4. Care should be taken to avoid splashing and well cross-contaminations.
5. All solutions contain sodium azide (≤0.09 %) as preservative. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Flow cytometer
- Calibrated adjustable precision single channel micropipettes (for volumes between 1 µL and 100 µL) with disposable tips
- Round or conical bottom microplates
- Microtubes
- Aluminum foil
- DMSO
- Peptides for new specificity tetramers

SYMBOL DEFINITIONS

= Consult Directions for Use
= Store Away From Direct Light
= Storage Temperature
= Amount
REF = Code Number
LOT = Lot Number
RUO = Research Use Only

TEST PROCEDURE

Carefully read this protocol before performing an assay. Bring all the reagents to room temperature prior to start and centrifuge briefly to pull liquid to the bottom of the tubes.

A. Generation of New Specificity Tetramer Using Peptide Exchange

Prior to performing the assay, bring to room temperature Peptide Exchange Factor and peptides to be used in the assay.

1. Dissolve each lyophilized peptide to be assayed in DMSO to a 10 mM solution (~10 mg/mL for a 9 amino acid peptide). (Note 1) Aliquots of this peptide solution can be further diluted in water to the desired concentration. For high affinity peptides, a 1 mM stock solution is a reasonable starting concentration for the assay. For lower affinity peptides, a higher concentration may be necessary, but may cause tetramer aggregation.

2. Pipet 50 µL of QuickSwitch™ Tetramer into a microtube or well of round- or conical-bottom 96 well microtiter plate.

3. Add 1 µL of peptide and mix gently with pipetting. (Note 2)

4. Add 1 µL of Peptide Exchange Factor from green capped vial and mix gently with pipetting.

5. Repeat steps 1-4 for each additional peptide.

6. Incubate at least for 4 hours at room temperature protected from light.

7. Tetramers are now ready for use in staining assays. (Notes 3 and 4)

8. Refrigerate tetramers at 2-8°C protected from light when not used.

Note that peptide exchange reaction volumes can be scaled up or down, so long as reagent proportions are maintained.

LIMITATIONS

1. The QuickSwitch™ Tetramer Kit has been devised mainly for exploratory research such as quickly determining presence/absence of an MHC/peptide specific $CD8^+$ T cell population in donor leukocytes. These tetramers are not intended to be a substitute for tetramers classically manufactured by folding of peptide with MHC and tetramerization with fluorochrome-conjugated streptavidin (Note 5).

2. Do not mix components from other kits and lots.

NOTES

Note 1. Most of peptides are soluble in DMSO. However some highly basic or acidic peptides may precipitate in DMSO and would require alternative buffers.

Note 2. The final peptide concentration is 20 µM in this assay. The user may want to test higher or lower peptide concentrations as well. Higher concentrations may increase the percentage of peptide exchange but have the risk to trigger tetramer aggregation. In some cases, working with concentrations lower than 20 µM may be beneficial.

Note 3. Tetramers obtained by peptide exchange are used directly for cell staining. However, the user may want to dialyze the tetramers to remove excess peptide, which may interfere with staining or cause tetramer aggregation.

Note 4. The QuickSwitch™ Tetramer concentration is 50 µg/mL, measured by MHC monomer content. Depending on the T cell receptor affinity towards the MHC/peptide complex, cell stainings require 0.5 ng to 2 µg tetramer per reaction.[5,6] MBL International recommends simultaneous staining of class I tetramer with anti-CD8 and other antibodies for 30 minutes at room temperature.

Note 5. To determine peptide exchange on tetramers, please use the QuickSwitch™ Quant Tetramer Kit.

REFERENCES

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6. Mielke S, Nunes R, Rezvani K, et al. 2008. *A clinical-scale selective allodepletion approach for the treatment of HLA-mismatched and matched donor-recipient pairs using expanded T lymphocytes as antigen-presenting cells and a TH9402-based photodepletion technique.* Blood. 111:4392-4402.

TRADEMARKS

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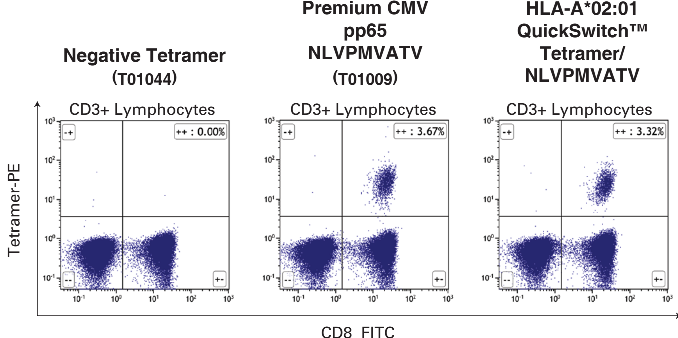
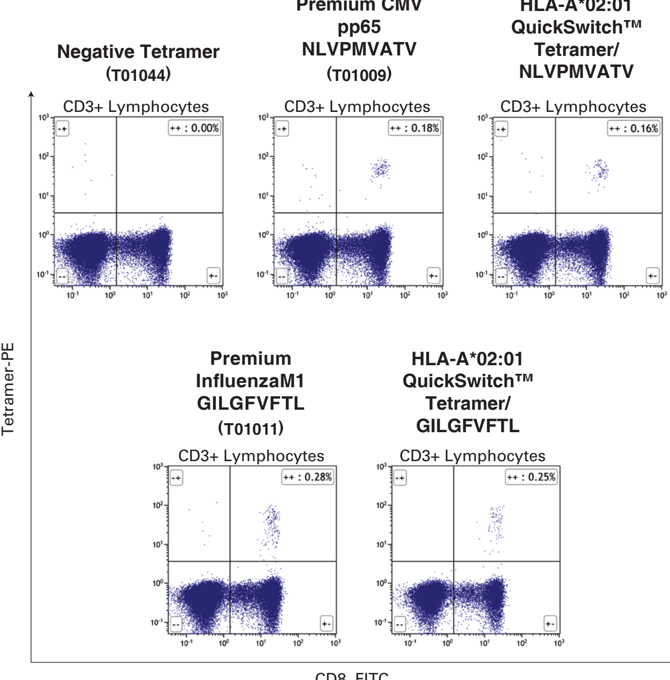
REPRESENTATIVE DATA

Fig. 1. Peptide-exchanged tetramers perform similarly to classically folded tetramers. Human PBMCs were stained with PE labeled HLA-A*02:01 tetramer, anti-CD3 PC5, and anti-CD8 (clone SFCI21Thy2D3)-FITC for 30 min at room temperature; acquired on FC500 flow cytometer (Beckman Coulter); and analysed using Kaluza Software (Beckman Coulter). Percent of antigen-specific T cells is shown for two different donors (A, B).

A. Donor 1



B. Donor 2



TROUBLESHOOTING TETRAMER STAINING

Problems	Potential Causes	Potential Solutions
High Background	Spillover from adjacent well(s) if exchange was performed in a plate	Use individual tubes instead of plate for exchange.
	Reagents contaminated	Store in a cool, dry place and do not pipet into vials.
	Peptide aggregation	Work with lower peptide concentrations.
	Aggregation	Perform doublet discrimination (e.g. plot FSC-H x FSC-A and gate on diagonal population representing single cell events).
		Dialyze tetramer.
		Decrease peptide concentration in exchange reaction.
	Incorrect compensation	Check compensation controls and resulting comp matrix.
	Impure T cell population	Perform positive gating (e.g. CD3, CD8) to identify T cells and/or incorporated a dump channel to eliminate non-T cells (e.g. B cell and monocyte markers).
	Contamination with dead cells	Use a viability dye and gate out dead/dying cells.
No Signal or Low Signal	MHC tetramer is too dilute or absent	Perform cross-titration of tetramer and CD8 antibody.
	Incorrect compensation and/or voltages set too low	Set voltages so that negative control is on scale, in the first decade. Check compensation controls and resulting comp matrix.
	Incorrect incubation times	Follow exactly the incubation times indicated in the protocol.
	Degraded reagent(s) are used in the assay	Make sure that all reagents are stored properly.
	No antigen-specific T cells present in sample	Obtain positive control target cells to verify tetramer staining (e.g. generate anti-gen-specific T cells using mixed lymphocyte peptide cultures method).