

Protocol for preparation and loading of U-Load® MHC II-peptide monomer onto U-Load Dextramer®

Background

U-Load® MHC II are peptide receptive molecules, which can be used to generate specific U-Load® MHC II-peptide monomer by loading your peptide of choice. The U-Load® MHC II-peptide monomer can easily be loaded onto fluorescently labeled U-Load Dextramer® and used to detect antigen-specific CD4⁺ T cells by flow cytometry. Optionally, the peptide-loaded monomer can be stored frozen at -80°C for later use. The U-Load® MHC II technology is highly flexible and suitable for screening of a single epitope in many samples as well as for screening large number of different epitopes in parallel.

Materials required

The materials listed in here are required for preparation of U-Load® MHC II-peptide monomer and U-Load Dextramer® MHC II.

U-Load® MHC II
U-Load Dextramer®
U-Load® MHC II loading buffer
U-Load® MHC II peptide loading component
U-Load Dextramer® dilution buffer

Materials required (not provided)

The materials listed in here are required for preparation of U-Load® MHC II-peptide monomer and U-Load Dextramer® MHC II.

Peptide of choice
DMSO (e.g., Sigma cat.# D2650)
PBS (pH 7.2-7.4) or ddH₂O

I. Preparation of U-Load® MHC II-peptide monomer

1. Thaw the U-Load® MHC II protein at 2-8°C or on ice.
2. Bring the other reagents to room temperature.
3. Dilute peptides of 10 mM stock solutions to 1 mM, e.g., by mixing 3 µL of peptide stock solution with 27 µL of PBS or ddH₂O.
4. Add 600 µL of U-Load® MHC II loading buffer to the vial containing the U-Load® MHC II peptide-loading component. Dissolve completely for 10 min at room temperature by gently turning the closed tube upside down every other minute.
5. To prepare U-Load® MHC II-peptide monomer, mix the reagents in Table A according to the listed sequence in a 1.5 mL tube. This will be enough to make 10, 20, or 50 tests U-Load Dextramer® MHC II.

Table A

Reagents	10 tests	20 tests	50 tests
Dissolved U-Load [®] MHC II peptide loading component	3 µL	4.5 µL	12 µL
Diluted peptide (1 mM)	2 µL	3 µL	8 µL
U-Load [®] MHC II (1 mg/ml)	5 µL	7.5 µL	20 µL
Total volume	10 µL	15 µL	40 µL

6. Mix the U-Load[®] MHC II-peptide monomer solution gently by pipetting up and down.
7. Cap the tube and centrifuge at 1000 x g for 1 min at room temperature to collect the mixture down.
8. Incubate the tube containing the U-Load[®] MHC II-peptide monomer solution at 37°C for 16-20 hours.
9. Centrifuge the tube at 1000 x g for 1 min at 4°C to collect the U-Load[®] MHC II-peptide monomer solutions down. Proceed to step 10 to make U-Load Dextramer[®] MHC II reagents. Alternatively, place your U-Load[®] MHC II-peptide monomer at -80°C for long-term storage.

II. Loading of U-Load Dextramer[®] MHC II

10. To load the U-Load[®] MHC II-peptide monomer onto U-Load Dextramer[®], mix the reagents in Table B in a 1.5 mL tube:
U-Load Dextramer[®] APC require different volume of reagents. See Procedural notes.

Table B

Reagents	10 tests	20 tests	50 tests
U-Load Dextramer [®] (PE/FITC)	20 µL	40 µL	100 µL
U-Load [®] MHC II-peptide monomer	7 µL	14 µL	4.5 µL
<i>incubate for 30 min at RT in the dark</i>			
U-Load Dextramer [®] Dilution Buffer	73 µL	146 µL	365.5 µL
Total volume U-Load Dextramer[®] MHC II	100 µL	200 µL	500 µL

11. Store the fluorescent U-Load Dextramer[®] MHC II reagent at 2-8°C in the dark until use.

III. Staining procedure

1. To analyze antigen-specific CD8⁺ T cells in blood using flow cytometry with a single specificity i.e only one type of U-Load Dextramer[®] MHC I, see www.immudex.com/Protocols/SingleReagent
2. If you intend to stain with multiple specificities i.e several different U-Load Dextramer[®] MHC I, see www.immudex.com/Protocols/MultipleReagents

Procedural notes

1. Protocol step 10: To assemble the U-Load® MHC II-peptide monomer with U-Load Dextramer® APC, mix the reagents in Table C in a 1.5 mL tube:

Table C

Reagents	10 tests	20 tests	50 tests
U-Load Dextramer® (APC)	20 µL	40 µL	100 µL
U-Load® MHC II-peptide monomer	4.5 µL	9 µL	23 µL
<i>incubate for 30 min at RT in the dark</i>			
U-Load Dextramer® Dilution Buffer	75.5 µL	151 µL	377 µL
Total volume U-Load Dextramer® MHC II	100 µL	200 µL	500 µL