

## Protocol for preparation and loading of U-Load<sup>®</sup> MHC II-peptide monomer onto U-Load Dextramer<sup>®</sup>

### Background

U-Load<sup>®</sup> MHC II are peptide receptive molecules, which can be used to generate specific U-Load<sup>®</sup> MHC II-peptide monomer by loading your peptide of choice. The U-Load<sup>®</sup> MHC II-peptide monomer can easily be loaded onto fluorescently labeled U-Load Dextramer<sup>®</sup> and used to detect antigen-specific CD4<sup>+</sup> T cells by flow cytometry. Optionally, the peptide-loaded monomer can be stored frozen at -80°C for later use. The U-Load<sup>®</sup> 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<sup>®</sup> MHC II-peptide monomer and U-Load Dextramer<sup>®</sup> MHC II.

U-Load<sup>®</sup> MHC II  
U-Load Dextramer<sup>®</sup>  
U-Load<sup>®</sup> MHC II loading buffer  
U-Load<sup>®</sup> MHC II peptide loading component  
U-Load Dextramer<sup>®</sup> dilution buffer

### Materials required (not provided)

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

Peptide of choice  
DMSO (e.g., Sigma cat.# D2650)  
PBS (pH 7.2-7.4) or ddH<sub>2</sub>O

### I. Preparation of U-Load<sup>®</sup> MHC II-peptide monomer

1. Thaw the U-Load<sup>®</sup> 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 ddH<sub>2</sub>O.
4. Add 600 µL of U-Load<sup>®</sup> MHC II loading buffer to the vial containing the U-Load<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> MHC II.

**Table A**

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

6. Mix the U-Load<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> MHC II-peptide monomer solutions down. Proceed to step 10 to make U-Load Dextramer<sup>®</sup> MHC II reagents. Alternatively, place your U-Load<sup>®</sup> MHC II-peptide monomer at -80°C for long-term storage.

## II. Loading of U-Load Dextramer<sup>®</sup> MHC II

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

**Table B**

Reagents	10 tests	20 tests	50 tests
U-Load Dextramer <sup>®</sup> (PE/FITC)	20 µL	40 µL	100 µL
U-Load <sup>®</sup> MHC II-peptide monomer	7 µL	14 µL	35 µL
<i>incubate for 30 min at RT in the dark</i>			
U-Load Dextramer <sup>®</sup> Dilution Buffer	73 µL	146 µL	365 µL
<b>Total volume U-Load Dextramer<sup>®</sup> MHC II</b>	100 µL	200 µL	500 µL

11. Store the fluorescent U-Load Dextramer<sup>®</sup> MHC II reagent at 2-8°C in the dark until use.

## III. Staining procedure

1. To analyze antigen-specific CD4<sup>+</sup> T cells in blood using flow cytometry with a single specificity i.e only one type of U-Load Dextramer<sup>®</sup> MHC II, see [www.immudex.com/Protocols/SingleReagent](http://www.immudex.com/Protocols/SingleReagent)
2. If you intend to stain with multiple specificities i.e several different U-Load Dextramer<sup>®</sup> MHC II, see [www.immudex.com/Protocols/MultipleReagents](http://www.immudex.com/Protocols/MultipleReagents)

**Procedural notes**

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

**Table C**

<b>Reagents</b>	<b>10 tests</b>	<b>20 tests</b>	<b>50 tests</b>
U-Load Dextramer <sup>®</sup> (APC)	20 µL	40 µL	100 µL
U-Load <sup>®</sup> 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 <sup>®</sup> Dilution Buffer	75.5 µL	151 µL	377 µL
<b>Total volume U-Load Dextramer<sup>®</sup> MHC II</b>	100 µL	200 µL	500 µL