

## 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<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® 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 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 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. no. D2650)  
 PBS (pH 7.2-7.4) or ddH<sub>2</sub>O

### 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 ddH<sub>2</sub>O.
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
<b>Total volume</b>	<b>10 µL</b>	<b>15 µL</b>	<b>40 µL</b>

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® (BV421, FITC, PE)	20 µL	40 µL	100 µL
U-Load® MHC II-peptide monomer	7 µL	14 µL	35 µL
<i>Incubate for 30 min at RT in the dark</i>			
U-Load Dextramer® Dilution Buffer	73 µL	146 µL	365 µL
<b>Total volume U-Load Dextramer® MHC II</b>	<b>100 µL</b>	<b>200 µL</b>	<b>500 µL</b>

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

## III. Staining procedure

12. To analyze antigen-specific CD4<sup>+</sup> T cells in blood using flow cytometry for one or more specificities using U-Load Dextramer® MHC II, see the MHC Dextramer® Staining Protocol at [immudex.com/resources/protocols](http://immudex.com/resources/protocols).

## 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**

Reagents	10 tests	20 tests	50 tests
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

## Trademarks

BV421 is equivalent to Brilliant Violet™ 421, which is a trademark or registered trademark of Becton, Dickinson and Company or its affiliates, and is used under license. Powered by BD Innovation.

## Label License

U-Load Dextramer<sup>®</sup> with BV421 is provided under an intellectual property license from Becton, Dickinson and Company. The purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in activity to generate revenue including (a) in manufacturing other than limited to quality control; (b) for therapeutic, diagnostic or prophylactic purposes; or (c) for resale, regardless of whether they are resold for use in research. For information on purchasing a license to this product for purposes other than research, contact Becton, Dickinson and Company, 10975 Torreyana Road, San Diego, California 92121 USA or [bdblicensing@bd.com](mailto:bdblicensing@bd.com).