

Protocol for Preparation of MHC II-peptide monomer and fluorescent U-Load Dextramer® MHC II

Background

U-Load MHC II are peptide receptive molecules, which can be used to generate specific MHC-peptide monomers by loading your choice of peptide. These monomers can easily be dextramerized with fluorescent labelled U-Load Dextramer® and used to detect antigen-specific CD4⁺ T cells in blood using a flow cytometry assay. Optionally the peptide-loaded monomers 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 a large number of samples as well as for screening large number of different epitopes in parallel.

Materials required

U-Load MHC II
 U-Load Dextramer® or U-Load dCODE Dextramer®
 Peptide
 DMSO (e.g. Sigma cat# D2650)
 PBS, pH 7.2-7.4 or ddH₂O

Preparation of MHC II-peptide monomer

1. Thaw the MHC II protein at 2-8°C or on ice.
2. Bring 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 minutes at room temperature by gently turning the closed tube upside down every other minute.
5. To prepare MHC-peptide monomer for dextramerization, mix the reagents in Table A below in the listed sequence in a 1.5 mL tube. This will be enough to make 10, 20 and 50 test U-Load Dextramer® MHC II, respectively.

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 (MHC II-peptide)	10 µL	15 µL	40 µL

6. Mix the MHC II-peptide monomer solution gently by pipetting up and down.
7. Cap the microtube and centrifuge at 1000xg for 1 minute at room temperature to collect the mixture down.
8. Incubate the microtube containing the MHC II-peptide monomer solution at 37°C for 16-20 hours.
9. Centrifuge the microtube at 1000xg for 1 minute at 4°C to collect the MHC II-peptide monomer solutions down. Proceed to step 10 to make U-Load

For research use only. Not for use in diagnostic or therapeutic procedures.

Dextramer[®] MHC II reagents or to step 12 to make U-Load dCODE Dextramer[®] MHC II reagents.

Alternatively place your MHC II-peptide monomers at -80°C for long-term storage.

Preparation of U-Load Dextramer[®] MHC II

10. To make 10, 20, or 50 tests of U-Load Dextramer[®] MHC II mix the reagents in Table B in the listed sequence in a 1.5 mL tube:

Table B

Reagents	10 tests	20 tests	50 tests
U-Load Dextramer [®]	20 µL	40 µL	100 µL
MHC II-peptide monomer	6.9 µL	13.8 µL	34.6 µL
<i>incubate for 30 min at RT in the dark</i>			
U-Load Dextramer [®] Dilution Buffer	73.1 µL	146.2 µL	365.4 µL
Total volume (U-Load Dextramer [®] MHC II)	100 µL	200 µL	500 µL

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

Staining protocol

To stain antigen-specific T cells follow the "General staining procedure for MHC II Dextramer[®] – PBMC's". (www.immudex.com/resources/protocols/)

Manufacturing of U-Load dCODE Dextramer[®] MHC II

12. To make 10, 20, or 50 tests of U-Load dCODE Dextramer[®] MHC II, mix the reagents in Table C in the listed sequence in a 1.5 mL tube:

Table C

Reagents	10 tests	20 tests	50 tests
U-Load dCODE Dextramer [®]	11.9 µL	23.7 µL	59.3 µL
MHC II-peptide monomer	6.9 µL	13.8 µL	34.6 µL
<i>incubate for 30 min at RT in the dark</i>			
U-Load dCODE Dextramer [®] Dilution Buffer	1.2 µL	2.5 µL	6.2 µL
Total volume (U-Load dCODE Dextramer [®] MHC II)	20 µL	40 µL	100 µL

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

Staining protocol

To stain antigen-specific T cells follow one of the protocols for dCODE Dextramer[®] reagents, that is compatible with the platform of either HiT, 10x compatible or RiO. (www.immudex.com/resources/protocols/)