

Protocol for preparation and loading of U-Load[®] MHC-II-peptide monomer onto U-Load dCODE 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 U-Load dCODE Dextramer[®] and used for characterization and quantification of antigen-specific T cells in a cell sample by next-generation sequencing (NGS) or single-cell multi-omics. U-Load dCODE Dextramer[®] is a DNA barcode labelled Dextramer[®] with a unique DNA barcode for each specificity. In addition, U-Load dCODE Dextramer[®] is labeled with PE for cell-sorting purposes. U-Load dCODE Dextramer[®] comes with DNA barcodes applicable for different applications:

- U-Load dCODE Dextramer[®] (HiT) - for epitope discovery, neoantigen screening, designed for multiplexing using PCR and NGS
- U-Load dCODE Dextramer[®] (RiO) for the detection of antigen-specific CD8+ or CD4+ T cells with additional information of gene expression, surface marker expression, and TCR sequence by single-cell multi-omics using the BD Rhapsody™ Single-Cell Analysis System
- U-Load dCODE Dextramer[®] (10x) for the detection of antigen-specific CD8+ and CD4+ T cells with additional information on gene expression, surface marker expression, and V(D)J sequences using the 10x Chromium™ platforms

The U-Load[®] MHC II and U-Load dCODE Dextramer[®] technologies are 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 MHC II-peptide monomer and U-Load Dextramer MHC II.

U-Load[®] MHC II
U-Load dCODE Dextramer[®] (HiT, RiO, 10x)
U-Load[®] MHC II loading buffer
U-Load[®] MHC II peptide loading component
U-Load dCODE 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 or 96-well U-bottom plate. The listed amounts will be enough to make 10, 20, or 50 tests of U-Load dCODE 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 dCODE 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 dCODE Dextramer[®] MHC II

10. To load the U-Load[®] MHC II-peptide monomer onto the U-Load dCODE Dextramer[®], mix the reagents in Table B in a 1.5 mL tube:

Table B

Reagents	10 tests	20 tests	50 tests
U-Load dCODE Dextramer [®]	12 µL	23.5 µL	59.5 µL
U-Load [®] MHC II-peptide monomer	7 µL	14 µL	34.5 µL
<i>incubate for 30 min at RT in the dark</i>			
U-Load dCODE Dextramer [®] Dilution Buffer	1 µL	2.5 µL	6 µL
Total volume U-Load dCODE Dextramer[®] MHC II	20 µL	40 µL	100 µL

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

III. Staining Procedures & Sequencing Workflows

- For U-Load dCODE Dextramer[®] (HiT): See www.immudex.com/Protocols/HiT
For U-Load dCODE Dextramer[®] (RiO): See www.immudex.com/Protocols/RiO
For U-Load dCODE Dextramer[®] (10x): See www.immudex.com/Protocols/10x