# Protocol for preparation and loading of easYmers® MHC I-peptide monomer onto U-Load dCODE Dextramer®

## Background

easYmers® powered by immunAware is a formulation of peptide-receptive MHC I monomer, which can be used to generate specific MHC I-peptide monomer by loading your peptide of choice. The easYmers® MHC I-peptide monomer can easily be loaded onto U-Load dCODE Dextramer® and used 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 easYmers® and U-Load dCODE Dextramer® technologies are highly flexible and suitable for screening of single epitopes in many samples or screening of large numbers of different epitopes in parallel. The easYmers® technology also allow evaluation of peptide binding to MHC I by assaying proper refolding of peptide-loaded monomer.

## Materials required

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

easYmers®
easYmers® loading buffer
easYmers® positive control peptide
U-Load dCODE Dextramer® (HiT, RiO, 10x)
U-Load dCODE Dextramer® dilution buffer

## Materials required (not provided)

The materials listed here are required for preparation of easYmers® MHC I-peptide monomer and U-Load dCODE Dextramer® MHC I and for the flow cytometry-based assay for evaluation of proper folding of easYmers® MHC I-peptide monomer.

Peptide of choice
DMSO (e.g., Sigma cat.# D2650)

Dilution buffer (PBS, 5% glycerol)
FACS buffer (PBS, 1% BSA (or FCS), 0.01% $NaN_3$)
Streptavidin-coated beads (Spherotech cat.# SVP-60-5)
Anti-human $\beta_2$m BBM.1-PE (Santa Cruz cat.# sc-13565 PE)

## Protocol steps and timing

Experimental workflow using the easYmers® and U-Load dCODE Dextramer® and estimated time to complete each step.


## I. Preparation of easYmers® MHC I-peptide monomer

1. Reconstitute your peptides of interest according to the manufacturer's instructions.
2. Dilute Peptide (easYmers® control peptide or peptide of interest) to 100 µM in $ddH_2O$. Keep on ice from this step on.
3. To prepare easYmers® MHC I-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 I.

   *Optional: To evaluate the peptide loading efficiency make a smaller volume of the easYmers® positive and the negative control (no peptide), i.e., easYmers® loaded with the included easYmers® positive control peptide or no peptide as listed in Table A.*

**Table A**

| **Reagents** | **10 tests** | **20 tests** | **50 tests** | **Positive Control** | **Negative control** |
|---|---|---|---|---|---|
| $ddH_2O$ | 3 µL | 6 µL | 15 µL | 2.5 µL | 3 µL |
| Peptide (100 µM) | 2 µL | 4 µL | 10 µL | 0.5 µL | - |
| easYmers® Loading Buffer | 5 µL | 10 µL | 25 µL | 3 µL | 3 µL |
| easYmers® (3 µM) | 20 µL | 40 µL | 100 µL | 3 µL | 3 µL |
| **Total volume of easYmers® MHC I-peptide monomer (2 µM)** | **30 µL** | **60 µL** | **150 µL** | **9 µL** | **9 µL** |

4. Mix by pipetting gently – *be careful not to form bubbles.*
5. Briefly centrifuge to collect all materials in the bottom of the tube and incubate at 18°C for 48 h.
6. Briefly centrifuge to collect all material in the bottom of the tube. 2 µM folded MHC I-peptide monomer are now ready for loading onto U-Load dCODE Dextramer® backbone or can be stored at -20°C for long-term storage.

7. Proceed to page 4 to evaluate peptide loading efficiency or continue to load onto U-Load dCODE Dextramer®.

## II. Loading of U-Load dCODE Dextramer® MHC I

8. To load the easYmers® MHC I-peptide monomer onto 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 |
|---|---|---|---|
| easYmers® MHC I-peptide monomer (2 µM) | 27 µL | 54 µL | 135 µL |
| U-Load dCODE Dextramer® (PE) | 12 µL | 24 µL | 60 µL |
| *incubate for 30 min at RT in the dark* | | | |
| U-Load dCODE Dextramer® Dilution Buffer | 11 µL | 22 µL | 55 µL |
| **Total volume U-Load dCODE Dextramer® MHC I** | **50 µL** | **100 µL** | **250 µL** |

9. Store the fluorescent U-Load dCODE Dextramer® MHC I 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

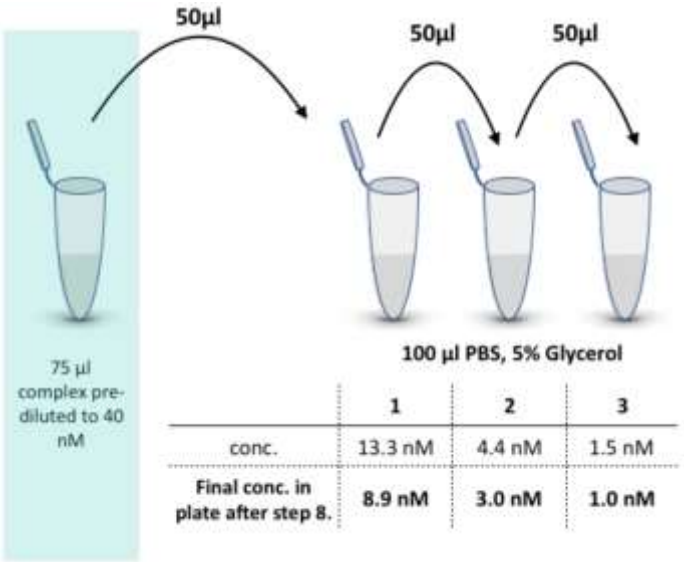
## Optional: Flow cytometry-based quality control assay for determination of peptide loading efficiency

### Background

After easYmers® MHC I-peptide monomerization (step 6 in the protocol), the relative peptide-loading efficiency can be determined by comparing your peptide of interest to the negative and positive loading controls using this assay. The negative loading control is empty easYmers® (no peptide). The positive loading control peptide is specific to and provided with the easYmers® you purchase. If this is your first time testing a particular easYmers® MHC I-peptide combination, this assay is highly recommended.

### Procedure: evaluation of easYmers® MHC I-peptide monomer formation

1. Prepare a sufficient volume of dilution buffer (PBS, 5% glycerol).
2. To determine the efficiency of the easYmers® MHC I-peptide folding take 3 µL of the prepared easYmers® MHC I-peptide monomer (1 µM) and dilute to 500 nM by adding 3 µL of dilution buffer.
3. Dilute each of the easYmers® MHC I-peptide monomer to give 75 µL of a 40 nM solution (e.g., for a 500 nM monomer: 6 µL folded monomer in 69 µL dilution buffer).
4. For all samples and positive and negative loading controls, transfer 50 µL of this pre-dilution (prepared in step 3) to the first tube. Make three subsequent serial 3-fold dilutions (50 µL in 100 µL dilution buffer), according to the figure below.




| | 1 | 2 | 3 |
|---|---|---|---|
| conc. | 13.3 nM | 4.4 nM | 1.5 nM |
| Final conc. in plate after step 8. | 8.9 nM | 3.0 nM | 1.0 nM |

5. Transfer 40 µL of each of these dilutions to the wells in a U-bottom shape 96-well plate, as suggested below. Also, prepare a background well (BLANK): 40 µL of dilution buffer (no beads or antibody will be added to this well).

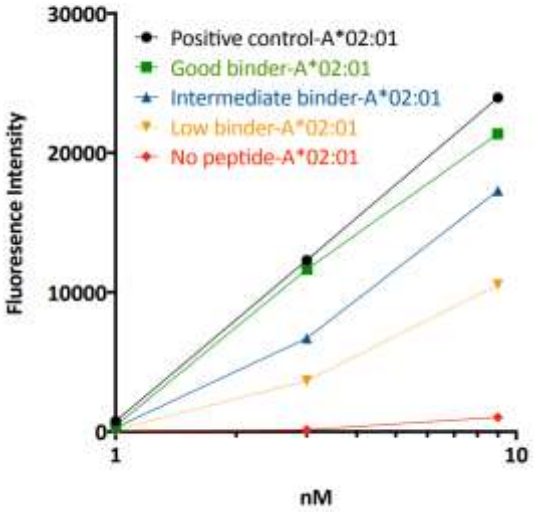
6. Prepare a sufficient volume of a 45-fold dilution of the streptavidin-coated beads in dilution buffer. Transfer 20 µL of the diluted bead suspension to each well.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BLANK | | P-1 | | S1-1 | | S3-1 | | S5-1 | | S7-1 | |
| B | | | P-2 | | S1-2 | | S3-2 | | S5-2 | | S7-2 | |
| C | | | P-3 | | S1-3 | | S3-3 | | S5-3 | | S7-3 | |
| D | | | | | | | | | | | | |
| E | | | N-1 | | S2-1 | | S4-1 | | S6-1 | | S8-1 | |
| F | | | N-2 | | S2-2 | | S4-2 | | S6-2 | | S8-2 | |
| G | | | N-3 | | S2-3 | | S4-3 | | S6-3 | | S8-3 | |
| H | | | | | | | | | | | | |

**BLANK :** No complex
**P1-3 :** Positive control dilutions (HLA with know peptide
**N1-3 :** Negative control dilutions (HLA without peptide)
**S1-S8 :** Sample dilutions (complexes to evaluate)

7. Mix well and seal the plates with sealing tape to avoid well to well contamination.
8. Incubate the plate on a rocking table at 37°C for 1 h.
9. Remove the sealing tape and wash by adding 160 µL FACS buffer.
10. Spin the plate at 700 x g for 3 min and discard the supernatant.
11. Resuspend the beads in 200 µL FACS buffer.
12. Spin the plate at 700 x g for 3 min and discard the supernatant.
13. Wash two more times by repeating step 10 and 12.
14. During the above washing steps, prepare a 200-fold dilution of the PE-labeled anti-human β2m monoclonal antibody BBM.1 in FACS buffer.
15. Resuspend the beads in 50 µL antibody solution per well.
16. Incubate the plate for 30 min at 4°C.
17. Wash by adding 150 µL FACS buffer. Spin the plate at 700 x g for 3 min and discard the supernatant.
18. Resuspend the beads in 200 µL FACS buffer. Spin the plate at 700 x g for 3 min and discard the supernatant.
19. Wash two more times by repeating step 17 and 18.
20. Resuspend the beads in 200 µL FACS buffer and analyze on a Flow cytometer.

## Example of the Flow cytometry-based assay:




**Flow cytometry-based detection of 4 different peptide-HLA-A*02:01 monomers**. MHC I-peptide monomer of A*02:01 and 4 different peptides, and a negative control (no peptide), were folded. CMV pp65 495-503 (NLVPMVATV) a known HLA-A*02:01 restricted epitope was used as positive control. The three other peptides are based on their A*02:01 binding stability categorized as good binder (T½ 6.5h), intermediate binder (T½ 3.5h), and low binder (T½ 0.7h). Three dilutions of the folded monomer were analysed in the flow cytometry-based assay. The X-axis gives the monomer concentration if complete folding is achieved.