# Protocol for Preparation of MHC I-Peptide Monomer and Fluorescent U-Load dCODE Dextramer® MHC I reagents

**Background**

easYmer® are highly active formulations of MHC I molecules, which can be used to generate specific MHC-peptide monomers by loading your own peptide of choice. The MHC-peptide monomers can easily be dextramerized with U-Load dCODE Dextramer® and used for profiling and quantification of antigen-specific T cells in cell sample. The U-Load dCODE Dextramer® reagent is a DNA barcode labelled Dextramer backbone with a unique barcode label for each specificity. U-Load dCODE Dextramer® reagent comes with labels applicable for different applications:

- U-Load dCODE Dextramer® (RiO) for multi-omics analysis of antigen-specific T cells, compatible with BD's Rhapsody single cell analysis platform
- U-Load dCODE Dextramer® (10x) – for multi-omics analysis of antigen-specific T cells, compatible with 10x Chromium Single Cell Gene Expression system
- U-Load dCODE Dextramer® (HiT) - for epitope discovery, neoantigen screening, designed for multiplexing using PCR and NGS,

The easYmer® and U-Load 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 easYmer® technology also allow evaluation of peptide binding to MHC I by assaying proper refolding of peptide loaded complexes.

**Materials required**

easYmer®
U-Load dCODE Dextramer® (RiO, 10x, HiT)
Peptide
DMSO (e.g., Sigma cat# D2650)
$ddH_2O$

**Preparation of MHC-peptide monomer**

1. Dilute Peptide (easYmer® Control Peptide or peptide of choice) to 100 µM in $ddH_2O$. Keep on ice from this step.
2. To prepare easYmer® MHC-peptide monomer for dextramerization, 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 reagent.
3. *Optional: To evaluate folding of peptide with MHC make a smaller volume of a positive and negative control, i.e., easYmer® loaded with the included control peptide and 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 | - |
| easYmer® Loading Buffer | 5 µL | 10 µL | 25 µL | 3 µL | 3 µL |
| easYmer® (3 µM) | 20 µL | 40 µL | 100 µL | 3 µL | 3 µL |

| Total volume | 30 µL | 60 µL | 150 µL | 9 µL | 9 µL |
|---|---|---|---|---|---|

4. Mix by pipetting gently – *be careful not to form bubbles.*
5. Make a short clearing spin to collect all materials in the bottom of the tube and incubate at 18°C for 48 h.
6. Make a short clearing spin to collect all material in the bottom of the tube. 2000 nM folded MHC-peptide monomer is now ready for further processing or placed at -20°C for long-term storage.
7. Proceed to step 8 to evaluate peptide binding or continue to dextramerization starting at step 9.
   To determine the efficiency of the easYmer® MHC-peptide folding take 2 µL of the prepared MHC-peptide monomer (2000 nM) and dilute to 500 nM by adding 6 µL of Loading buffer. Then follow the "Protocol for Flow cytometry-based assay of peptide- easYmer® MHC I complex formation" (page 3).

**Preparation of U-Load dCODE Dextramer® MHC I**

8. To dextramerize the peptide-loaded easYmer® MHC monomers (MHC-peptide monomer) with U-Load dCODE Dextramer® reagent, mix the reagents in Table B in a 1.5 mL tube:

**Table B**

| Reagents | 10 tests | 20 tests | 50 tests |
|---|---|---|---|
| MHC-peptide monomer (2 µM) | 27 µL | 54 µL | 135 µL |
| U-Load dCODE Dextramer® | 12 µL | 24 µL | 60 µL |
| *incubate for 30 min at RT in the dark* | | | |
| U-Load dCODE Dextramer®<br>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 DNA barcode labelled U-Load dCODE Dextramer® MHC I reagents at 2-8°C in the dark until use. *5 µL per test is recommended for staining of 1-10 x $10^6$ PBMC.*

**Analysis of antigen-specific T cells**

Antigen-specific T cells can be analyzed on three sequencing platforms:

- U-Load dCODE Dextramer® (HiT) for epitope discovery, neoantigen screening and immune monitoring follow the protocol "dCODE Dextramer® (HiT) Package Insert"
- U-Load dCODE Dextramer® (10x) for multi-omics analysis of antigen-specific T cells and TCR sequencing using 10x Chromium Single Cell Gene Expression follow the protocol "dCODE Dextramer® (10x compatible) Staining Protocol"
- U-Load dCODE Dextramer® (RiO) for multi-omics analysis of antigen-specific T cells and gene expression profiling using the BD Rhapsody™ Single-Cell Analysis System follow the protocol "dCODE Dextramer® (RiO) Package Insert" (https://www.immudex.com/resources/protocols/)

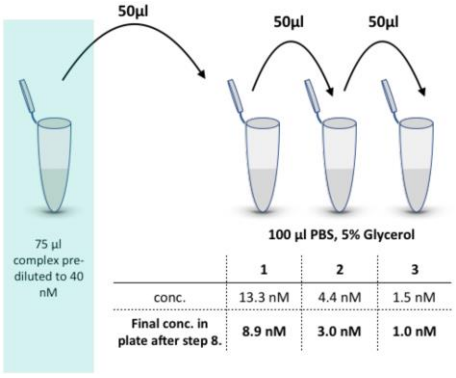
# Flow Cytometry-Based Assay of Peptide-MHC I Complex Formation

**Background**

After completed peptide loading of easYmer® monomer (step 8 in the protocol "Preparation of MHC I-peptide monomer and fluorescent U-Load Dextramer® MHC I"), proper folding of MHC-peptide monomers can be evaluated using the assay described in this procedure.

**Procedure: evaluation of peptide-MHC I complex formation**

1. The MHC-peptide monomers to be assayed are prepared according to protocol: "Preparation of MHC I-peptide monomer and fluorescent U-Load Dextramer® MHC I". The peptide loading setup should include a positive control (provided with the easYmer®): a peptide that is known to support folding of the MHC molecule of interest, and a negative control: without peptide.
2. Prepare sufficient dilution buffer (PBS, 5% glycerol) for the whole assay.
3. Dilute each of the MHC-peptide monomers to give 75 µL of a 40 nM solution (e.g., for a 500 nM complex: 6 µL folded complex in 69 µL dilution buffer).
4. For all samples and positive and negative 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): transfer 40 µL of dilution buffer
6. Prepare a sufficient volume of a 45-fold dilution of the streptavidin-coated beads (6-8um; SVP-60-5) in dilution buffer. Transfer 20 µL of the diluted bead suspension to each well.

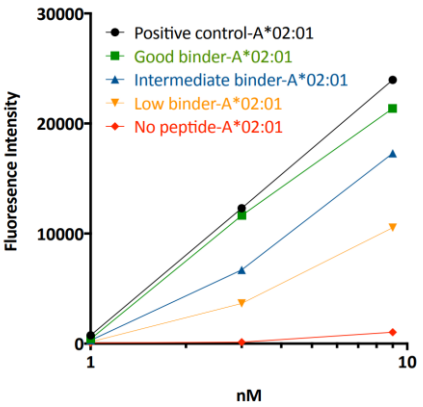
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BLANK | | P-1 | | S1-1 | | S3-1 | | S5-1 | | S7-1 | |
| B | | | P-2 | | S1-2 | | S3-2 | | S5-2 | | S7-2 | |
| C | | | P-3 | | S1-3 | | S3-3 | | S5-3 | | S7-3 | |
| D | | | | | | | | | | | | |
| 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 1h.
9. Remove the Sealing Tape and wash by adding 160 µL FACS buffer.
10. Spin the plate at 700xg for 3 min and flip out the supernatant.
11. Resuspend the beads in 200 µL FACS buffer.
12. Spin the plate at 700xg for 3 min and flip out the supernatant.
13. Wash two more times by repeating step 11. 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.

    Wash by adding 150 µL FACS buffer. Spin the plate at 700g for 3 min and flip out the supernatant.
17. Resuspend the beads in 200 µL FACS buffer. Spin the plate at 700xg for 3 min and flip out the supernatant.
18. Wash two more times by repeating step 17. and 18.
19. 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-peptide monomers 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 complexes were analysed in the flow cytometry-based assay. The X-axis gives the complex concentration if complete folding is achieved.