

Assay background

The easYmer kit features a highly active formulation of HLA class I (HLA-I) molecules, which can be used to generate specific peptide-HLA class I monomers of your choice in your own laboratory. These monomers can easily be tetramerized with fluorophore conjugated streptavidin and used to stain antigen specific T cells for analysis in flow cytometric assays. Optionally, the monomers can be stored frozen for later use. The easYmer technology is highly flexible and suitable for screening of a single epitope in a large number of samples, as well as for screening of a large number of different epitopes in parallel.

easYmer kit

The easYmer kit contains a peptide receptive preparation of HLA, a folding buffer, a positive control peptide for HLA-complex folding. The exact identities of these components are given in the product specification sheet

Additional materials and equipment required

Fluorophore-conjugated streptavidin e.g.: *Streptavidin-PE* (BD; Cat# 554061); *Streptavidin-APC* (BD; Cat#554067); *Streptavidin-BV421* (BD; Cat# 563259)

DMSO (e.g. Sigma cat# D2650)

ddH₂O

Sealing Tape (Thermo Scientific Nunc™ cat# 236366)

FACS buffer: PBS with 1%BSA (or FCS) and 0.1% NaN₃.

Vortex mixer

96-well U-bottom plate

Centrifuge with a plate rotor

Flow cytometer

Recommendations

The easYmer kit cannot generate monomers unless the underlying peptide-MHC-I interaction is a productive one, i.e. that it is of reasonable affinity and stability. We recommend that you use predictors (e.g. one from the [NetMHC series](#)) to assist you in the design of suitable monomers. We strongly recommend that you experimentally validate the interaction(s) of the peptide-MHC-I interaction(s) of your choice. To this end, the easYmer kit includes a simple protocol, *Flow cytometry-based assay of peptide-HLA-I complex formation*, which allows you to examine proper peptide-MHC class I interaction.

Protocol for small-scale production of many monomers in parallel

Generation of small-scale peptide-HLA complexes

1. Work on ice
2. Dilute the positive control peptide and the peptide(s) of choice to 100µM in ddH₂O.
 - Peptide 1mM stock: 3 µl of peptide stock + 27 µl ddH₂O (positive control)
3. Make 90 µL peptide-HLA complex. This should be enough to validate folding and produce tetramers sufficient for at least 5 tests.
4. Make a smaller volume of a positive and negative folding controls, the included peptide and no peptide, respectively.
5. Per well, add the following reagents in a 96-well U-bottom plate:

Reagents	5 tests	Positive Control	Negative Control
ddH ₂ O	57 µL	15 µL	16 µL
Peptide (100µM)	3 µL	1 µL	
FoldingBuffer (X6)	15 µL	4 µL	4 µL
easYmer	15 µL	4 µL	4 µL
Total volume	90 µL	24 µL	24 µL

6. Mix by pipetting up and down. Assure a thorough mixing – be careful not to form bubbles.

Tip: to avoid mixing of samples make an empty well spacing between samples.
7. Seal the plates with Sealing Tape and incubate at 18°C for 48h.
8. When using optimal binding peptides, 90µL with 500nM folded monomer will be obtained.
9. To validate and evaluate the efficiency of the folding follow the protocol for “*Flow cytometry-based assay of peptide-HLA-I complex formation*”.

Production of Tetramer

1. Transfer 60 µL of each sample into a new 96-well U-bottom plate.
2. To tetramerize 60 µL of 500nM complexes use the equivalent of 2.4 µL of a 0.2 mg/ml Streptavidin-fluorophore.

It is recommended to use either of the following:

- 1µL Streptavidin-PE (BD; Cat# 554061; 0.5 mg/ml),
- 2.4µL Streptavidin-APC (BD; Cat#554067; 0.2 mg/ml),
- 4.8µL Streptavidin-BV421 (BD; Cat# 563259; 0.1 mg/ml)

3. Add the Streptavidin-fluorophore and mix thoroughly. Seal the plate and incubate at 4°C in the dark for at least 1h.
4. To analyze for antigen specific CD8+ T cells follow the protocol for “*HLA class I tetramer staining of human T cells*”.

Protocol for large-scale production of one or a few monomers

Generation of large-scale peptide-HLA complex

One tetramer enough for 50 tests

1. Work on ice
2. Dilute the peptide to 100 μM in ddH₂O.
3. Make 540 μL peptide-HLA complex (this should be enough to validate folding and produce tetramers sufficient for at least 50 tests)
4. To evaluate folding, make a smaller volume of a positive and negative control, the included peptide and no peptide, respectively.
5. In a 1.5-2mL tube add the following reagents:

Reagents	50 tests	Positive Control	Negative Control
ddH ₂ O	343 μL	15 μL	16 μL
Peptide (100μM)	17 μL	1 μL	
Folding Buffer (X6)	90 μL	4 μL	4 μL
easYmer	90 μL	4 μL	4 μL
Total volume	540 μL	24 μL	24 μL

6. Mix by vortexing the tube.
7. Incubate at 18°C for 48h.
8. When using optimal binding peptides, 540μL with 500nM folded monomer will be obtained.
9. To validate and evaluate the efficiency of the folding follow the protocol for *Flow cytometry-based assay of peptide-HLA-I complex formation*.

Production of Tetramer

5. The monomer production or aliquots hereof can be tetramerized.
6. Transfer 500μL of each sample into a new tube.
7. To tetramerize 500μL of the monomer complexes use the equivalent of 20μL of a 0.2mg/ml Streptavidin-fluorophore.

It is recommended to use either of the following:

- 8μL Streptavidin-PE (BD; Cat# 554061; 0.5mg/ml)
- 20μL Streptavidin-APC (BD; Cat#554067; 0.2mg/ml)
- 40μL Streptavidin-BV421 (BD; Cat# 563259; 0.1mg/ml)

8. Add the Streptavidin-fluorophore volume in 3 steps:
 1. Add ⅓ of the streptavidin volume mix and incubate in the dark for 10 min. at 4°C
 2. Add ⅓ of the streptavidin volume mix and incubate in the dark for 10 min. at 4°C
 3. Add ⅓ of the streptavidin volume mix and incubate in the dark for 30 min. at 4°C
9. The tetramer is now ready for use, and can be stored at 4°C for several months.
10. To analyze for antigen specific CD8+ T cells follow the protocol for “*HLA class I tetramer staining of human T cells*”.