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PRODUCT SHEET

ProVE® *SL* Self-loading MHC Class I Tetramer, APC labelled:

- T000AA-4X- to T000CE-4X-

ProVE® *SL* Self-loading Recombinant MHC Class I Tetramer for Peptide Loading:	ProVE® *SL* Self-loading MHC Class I Tetramers are provided ready for peptide addition. The desired peptide can be loaded into the peptide binding groove and the resultant APC labelled MHC Class I-peptide complexes used to bind to CD8+ T cell receptors of a particular specificity in order to identify specific CD8+ T cells in flow cytometry. **For Research Use Only. Not for use in therapeutic or diagnostic procedures**.
Test size:	ProVE® *SL* Self-loading MHC Class I Tetramers (APC labelled) are provided in 50 test and 150 test sizes
Test Volume:	2 µl / test.
Test Specification:	One test contains sufficient reagent to stain approximately 1-2 x10^6 cells. We recommend that the user titrate the reagent to determine the optimum amount to use in their specific application.
Concentration/ Formulation:	ProVE® *SL* Self-loading MHC Class I Tetramers (APC-labelled) are supplied at a concentration of approximately 0.2 mg/mL in PBS, containing 1% animal free protein stabilizer and 0.025% sodium azide.
Storage Condition:	4ºC. Protect from light. **Do not freeze.**
Shelf Life:	The tetramer is stable for 6 months if stored as instructed above.
Fluorochrome:	Allophycocyanin (APC): excites at 650nm; emits at 600nm
Hazards:	This reagent is formulated in 0.025% sodium azide. Under acidic conditions the toxic compound hydrazoic acid may be released. Compounds containing sodium azide should be flushed with running water while being discarded.

Quality Control Assay Results

Appearance: Clear, pale blue solution

Protein Characterization: Passed

Released by:
(Date as per product label above)

Peptide Loading Protocol

1. Transfer 50 tests (100 µl) Empty Class I MHC tetramer to a fresh microtube and centrifuge at 10,000 ×*g* for 5 minutes in a chilled microcentrifuge to remove aggregates.
2. Reconstitute your selected peptide in dimethyl sulfoxide (DMSO) at a concentration of 10-50 mM.
3. Dilute the Empty Class I MHC tetramer 1 in 5 with PBS. This ensures that the final concentration of DMSO in the reaction mix is not greater than 1%.
4. Add the peptide to the Empty Class I MHC tetramer at a final concentration of 80 µM. This should be no less than a 1 in 125 dilution of your peptide stock. Mix well by pipetting up and down and leave at room temperature for 24 hours, protected from light.

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5. After 24 hours, centrifuge the peptide-loaded MHC tetramer at 10,000 ×*g* for 5 minutes in a chilled microcentrifuge and store at 4ºC.

The optimal loading protocol will depend on the properties of the peptide to be loaded. The protocol given here is therefore only supplied as a guide and researchers are advised to investigate optimal conditions for ligand loading.

We recommend loading the tetramer with peptide immediately prior to staining. Stability of the loaded tetramer will depend on the specific peptide used.

Peptide Loading Worked Example

Loading 50 tests of tetramer with peptide

Note: This protocol assumes a peptide molecular weight of approximately **1,000 Da (1 kDa)**. If your peptide has a substantially different molecular weight, adjust the volume added to maintain the same molar amount of peptide.

1. Transfer 100 µl (50 tests) of Empty Class I MHC tetramer to a fresh microtube and centrifuge at 10,000 ×g for 5 minutes in a chilled microcentrifuge to remove aggregates.
2. Reconstitute 1 mg of your selected peptide in 100 µl dimethyl sulfoxide (DMSO).
    a. If your peptide purity is <100%, add the correct volume of DMSO for a 0.1mg/ml final concentration. E.g. if your peptide is 80% pure, reconstitute 1mg of peptide in 80ul of DMSO
3. Dilute 100 µl Empty Class I MHC tetramer with 400µl PBS. This ensures that the final concentration of DMSO in the reaction mix is not greater than 1%.
4. Add 4 µl of the reconstituted peptide to the 500 µl Empty Class I MHC tetramer. Mix well by pipetting up and down and leave at room temperature for 24 hours, protected from light.
5. After 24 hours, centrifuge the peptide-loaded MHC tetramer at 10,000 ×*g* for 5 minutes in a chilled microcentrifuge and store at 4ºC.

Cellular Staining Protocol

Additional materials required: Wash Buffer (0.1% sodium azide, 0.1% BSA in PBS), Fix Solution (1% fetal calf serum, 2.5% formaldehyde in PBS), anti-CD8 antibody, anti-CD19 antibody.

1. Centrifuge all tetramers in a chilled microcentrifuge at 10,000 ×*g* for 5 minutes. This will remove protein aggregates that contribute to non-specific staining. Maintain reagents on ice, shielded from light until required. Do not aspirate any part of the pelleted aggregates when removing material for staining.
2. Allocate 1-2 × 10^6 lymphoid cells (PBMCs) per staining condition. Allocate only 2-5 × 10^5 cells per staining condition when using cell lines or clones, due to the higher frequency of antigen-specific T cells.
3. Wash the cells with 2 ml Wash Buffer and resuspend in the residual volume (~50 µl).
4. Add one test (~0.4 µg, 10ul) of APC labelled loaded Class I MHC tetramer to the cells and mix by pipetting.
5. Incubate at RT for 30 minutes, shielded from light.
6. Wash the cells with 2 ml Wash Buffer and resuspend them in the residual volume.
7. Add an optimally titrated amount of anti-CD8 antibody per staining condition. At this stage, it is recommended to add anti-CD19 antibody in order to exclude non-specific staining of B cells from the cytometry analysis.
8. Incubate samples on ice for 20-30 minutes, shielded from light.
9. Wash the cells twice with 2 ml Wash Buffer and resuspend thoroughly before adding 200 µl Fix Solution. Store them in Fix Solution in the dark until analysis.

Protocol Optimization

For further tips on protocol optimization refer to http://www.proimmune.com/support-protocol-optimization

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