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PRODUCT SHEET

ProVE® *SL* Self-loading MHC Class I Monomer, empty Biotin-labelled:

- M000-AA to M000-CD

ProVE® *SL* Self-loading Recombinant MHC Class I Monomer for Peptide Loading:	ProVE® *SL* Self-loading MHC Class I Monomers are provided ready for peptide addition. The desired peptide can be loaded into the peptide binding groove and the resultant MHC Class I-peptide complexes used to bind to CD8+ T cell receptors of a particular specificity. Biotin-labelled MHC loaded with a single peptide species may be combined with fluorescent-labelled streptavidin to produce MHC tetramers for the purpose of identifying of specific CD8+ T cells in flow cytometry. In addition, peptide-loaded biotin-labelled MHC Class I monomers can be used to isolate (or deplete) antigen specific CD8+ T-cells through the use of streptavidin-coated magnetic microbeads. Isolation of antigen-specific T cells in this manner is useful if viable cells are needed for downstream applications. Peptide-loaded biotin-labelled MHC Class I monomers can also be immobilised on streptavidin-coated surfaces for use in plate-based assays such as ELISA. **For Research Use Only. Not for use in therapeutic or diagnostic procedures**.
Test size:	ProVE® *SL* Self-loading MHC Class I Monomers are provided in 35 µg and 100 µg sizes
Concentration/ Formulation:	ProVE® *SL* Self-loading MHC Class I Monomers (biotin-labelled) are supplied at a concentration of approximately 0.4 mg/mL in PBS, stabilized with 1% BSA and 0.025% sodium azide.
Storage Condition:	Use liquid nitrogen to flash-freeze upon receipt and store at -80 ºC. **Avoid freeze-thaw cycles.**
Shelf Life:	The monomer is stable for 6 months if stored as instructed above.
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: Colorless solution

Protein Characterization: Passed

Released by:
(Date as per product label above)

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Peptide Loading Protocol

1. Transfer 35 µg Empty Class I MHC monomer 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. Add the peptide to the Empty Class I MHC material 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. Store out of direct sunlight.
4. After 24 hours, centrifuge the peptide-loaded MHC at 10,000 ×*g* for 5 minutes in a chilled microcentrifuge and store at 4ºC for up to 3 months.

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.

Tetramerization Protocol

1. Monomers may be conjugated to fluorophore-labelled streptavidin at approximately 4 to 1 molar ratio of monomer to streptavidin. The monomer is provided at a concentration of ~8 µM. 0.5 µg of tetramer is sufficient to stain 1-2 × 10^6 lymphoid cells (PBMCs).
2. Centrifuge the loaded Class I MHC at 10,000 ×*g* for 5 minutes in a chilled microcentrifuge.
3. Calculate the volume of fluorophore-conjugated streptavidin needed for tetramerization.
4. Add one fifth of the total required volume of streptavidin to the peptide-loaded Class I MHC. Incubate at 4ºC for 15 minutes and then proceed to add another fifth-volume to the mixture. Continue adding more streptavidin at 15-minute intervals until all the streptavidin has been added.
5. To maintain product stability it is advisable to add BSA to 1% final volume with PBS/0.025% sodium azide.
6. Store at 4ºC for up to 3 months.

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 (~0.5 µg) of fluorescent 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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