PROIMMUNE
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PRODUCT SHEET

Pro5® Pentamer Biotin-labeled:

- F1X-
- F81X-
- Fnnn-1X- (but not FN01 or F712)
- Fnnn-81X- (but not FN01 or F712)

*for 81X, also require PS_A003-3 or A502-3

Pro5® Recombinant MHC Pentamer:	Biotin-labeled Pro5® MHC Class I Pentamers are used to identify antigen-specific $CD8^+$ T lymphocytes. Multimeric MHC-peptide complexes bind to T cell receptors (TCRs) of a particular specificity (as determined by the MHC allele and peptide combination). $CD8^+$ T cells stained with biotin-labeled Pro5® MHC Pentamers, followed by fluorescent streptavidin secondary reagent, can be analyzed by flow cytometry to determine the frequency of antigen-specific T cells. Biotin-labeled Pro5® Pentamers can also 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 further manipulation, such as T cell culture or gene expression profiling. Biotin-labeled Pro5® MHC Pentamers can also be used in plate-based assays, such as ELISA, where they can be immobilized on streptavidin-coated surfaces. **For Research Use Only. Not for use in therapeutic or diagnostic procedures**.
Test Volume:	10 µl / test.
Test Specification:	One test contains sufficient reagent to stain approximately 1×10^6 cells. We recommend that the user titrate the reagent to determine the optimum amount to use in their specific application.
Concentration/ Formulation:	The Pro5® Pentamer concentration is approximately 0.05 mg/ml in PBS, stabilized with 1% BSA and 0.025% sodium azide.
Storage Condition:	4°C for 6 months or -80°C for at least 12 months. **Avoid freeze-thaw cycles.**
Shelf Life:	12 months if stored at -80°C.
Hazards:	This reagent is formulated in 0.025% sodium azide. Under acid conditions the toxic compound hydrazoic acid may be released. Compounds containing sodium azide should be flushed with running water while being discarded.

Cellular Staining Protocol (Figure 1)

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 (optional†).

1. Centrifuge Pro5® MHC Pentamer in a chilled microcentrifuge at 14,000 ×*g* for 5 minutes. This will remove protein aggregates that contribute to non-specific staining.
2. Allocate 1-2 × 10^6 lymphoid cells (PBMCs or splenocytes) per staining condition. Allocate only 2-5 × 10^5 cells per staining condition when using T cell clones or lines, due to the higher frequency of antigen-specific T cells.
3. Wash the cells with 2 ml Wash Buffer and resuspend them in the residual volume (~50 µl). Keep tubes chilled on ice for all subsequent steps, except where indicated.
4. Add one test (10 µl) of biotin-labeled Pro5® MHC Pentamer to the cells and mix well.
5. Incubate at room temperature (22°C) for 10 minutes.
6. Wash the cells with 2 ml Wash Buffer and resuspend them in the residual volume.
7. Add fluorescent-labeled streptavidin, anti-CD8 and anti-CD19 antibodies to the cells and mix well. (†Use of the anti-CD19 antibody is recommended in order to exclude non-specific staining of B cells from your 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.

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The Pentamer-positive cells are most conveniently viewed by gating first on live, CD19-negative lymphoid cells and then analyzing on a two-color plot showing CD8 on the *x*-axis and Pentamer on the *y*-axis.

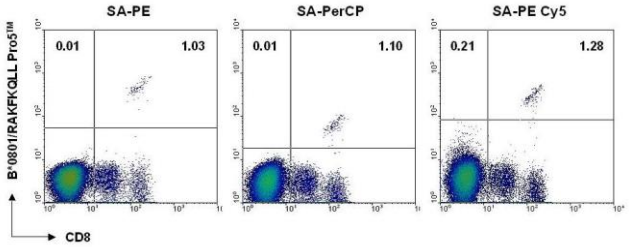

Figure 1: *Antigen-specific T cells were identified using a biotin-labeled Pro5® MHC Pentamer followed by streptavidin conjugated to R-PE, PerCP or PE-Cy5, as detailed in the protocol. The figure above demonstrates that a variety of SA fluorochromes can be used in conjunction with biotin-labeled Pentamer staining, to visualize a clear population of antigen-specific cells.*

Bead Isolation Protocol (Figure 2)

Additional materials required: Wash Buffer (0.1% sodium azide, 0.1% BSA in PBS), Streptavidin-coated magnetic microbeads.

1. For best results start with at least 1×10^7 lymphoid cells (PBMCs or splenocytes).
2. Wash the cells with Wash Buffer and resuspend them in 200 µl Wash Buffer.
3. Add 1 test (10 µl) biotin-labeled Pro5® MHC Pentamer per 2×10^6 cells and mix well.
4. Incubate at room temperature (22°C) for 10 minutes.
5. Wash the cells and resuspend them in 500 µl Wash Buffer.
6. Add an appropriate quantity of streptavidin beads, according to the manufacturer's instructions.
7. Incubate on ice for 30 minutes with mixing.
8. Bring the volume in the tube up to 2 ml with Wash Buffer then place in a magnetic particle separator.
9. Leave for 3-5 minutes. Wash the fraction containing bead:cell complexes 3 times with Wash Buffer before use.

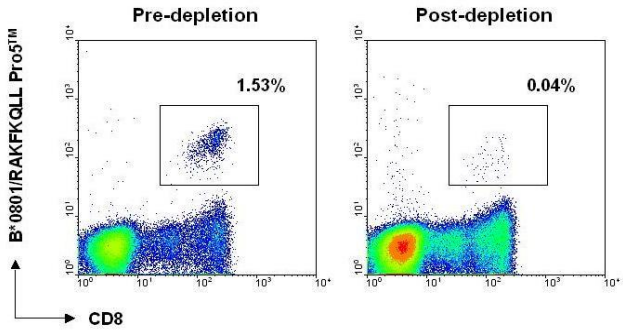

Figure 2: *The left panel clearly identifies a population of antigen-specific CD8+ T cells present in a cell sample. Following magnetic bead cell isolation with biotin-labeled Pro5® MHC Pentamer, the right panel shows that successful depletion of the antigen-specific cells has resulted in a significantly reduced Pentamer-positive population.*

Microplate Immobilization Protocol

Additional materials required: PBS, streptavidin, Coating Buffer (0.1 M $NaHCO_3$, pH 8.2), Wash Buffer (0.05% Tween-20 in PBS), Blocking Buffer (5% BSA in PBS).

1. Coat an ELISA plate with 100 µl per well of 1 µg/ml streptavidin in 0.1 M $NaHCO_3$, pH 8.2. Incubate the plate overnight at 4ºC
2. Wash wells 3 times with Wash Buffer
3. Add 200 µl Blocking Buffer per well and incubate 1 hour at room temperature.
4. Wash wells 3 times with Wash Buffer.
5. Add 50 ng per well biotin-labeled Pro5® MHC Pentamer and incubate 1 hour at room temperature.
6. Wash wells 3 times with Wash Buffer and proceed with your assay.

Protocol Optimization

These protocols may require some optimization since the binding affinity of the MHC molecule for the TCR varies depending on the allele/peptide combination. We recommend titrating all reagents to determine the optimum quantities required.

Quality Control Assay Results

Appearance: Clear, colorless solution

Protein Characterization: Passed

Released by:
(Date as per product label above)

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