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Product Sheet
Pro5® Recombinant MHC Pentamer Pre-labeled with Biotin

Biotin-labeled Pro5® Recombinant MHC Pentamer:	Biotin-labeled Pro5® MHC class I Pentamers allow the enumeration and isolation of 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® Pentamers, followed by staining with fluorescent streptavidin conjugates, can be analyzed by flow cytometric analysis in order 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 need to be obtained for further analysis such as T cell culture or gene expression profiling. Biotin-labeled Pro5® MHC class I Pentamers can also be used in plate base assays such as ELISAs where they can be immobilized to streptavidin coated surfaces. **For Research Use Only. Not for use in therapeutic or diagnostic procedures.**
Test specification:	One test is sufficient to stain approximately 1×10^6 cells. Less reagent may be adequate, and it is recommended that the customer determines the optimum amount appropriate for each application.
Test volume:	10 µl / test
Concentration/ Formulation:	The Pro5® Pentamer concentration is approximately 0.05 mg/ml in PBS stabilized with 1% BSA and 0.01% sodium azide.
Storage Conditions:	4°C for up to 6 months. -80°C for longer stability. **Avoid freeze-thaw cycles.**
Shelf Life:	6 months if stored as instructed above.
Hazards:	This reagent is formulated in 0.01% sodium azide. Under acid conditions the toxic compound hydrazoic acid may be released. Solutions containing sodium azide should be flushed with running water while being discarded.

Flow Cytometry Protocol (Figure 1)

Materials required Wash buffer (0.1% sodium azide, 0.1% BSA in PBS), Fix solution (1% fetal calf serum, 2.5% formaldehyde in PBS), Biotin-labeled Pro5® Pentamer specific for the antigen of choice, Fluorescent-labeled streptavidin conjugate, Anti-CD8 antibody (of a different fluorescence to the streptavidin conjugate).

1. Allocate 1-2 × 10^6 lymphoid cells (PBMC 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 high frequency of antigen-specific T cells).
2. Wash the cells with 2 ml wash buffer, spin down (500 x *g* for 5 minutes), discard supernatant and resuspend in the residual liquid (~ 50µl).
3. Add one test (10 µl) of biotin-labeled Pentamer to the cells and mix by pipetting.
4. Incubate at room temperature (22ºC) for 10 minutes.
5. Wash cells with 2 ml wash buffer, as for step 2, and resuspend in the residual liquid (~ 50µl).
6. Add an optimal amount of labeled streptavidin and anti-CD8 antibody (and any other secondary antibodies) and mix by pipetting.
7. Incubate on ice for 20 minutes, shielded from light.
8. Wash the cells twice with 2 ml wash buffer, as for step 2, and resuspend cells in 200 µl fix solution. Store in the refrigerator in the dark until flow cytometric analysis. The Pentamer-positive cells are most conveniently viewed by gating first on live lymphoid cells and then analyzing on a two-color plot showing CD8 on the x-axis and Pentamer on the y-axis.

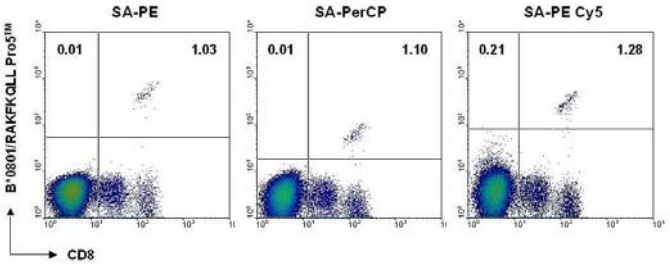

Figure 1: 1 x 10^6 peripheral blood cells were stained using a B*0801/RAKFKQLL (EBV BZLF-1)-specific biotin-labeled Pro5® Pentamer followed by either SA-PE, SA-PerCP, or SA-PE Cy5, as detailed in the protocol. The figure demonstrates that a variety of SA-fluorochromes can be used in conjunction with biotin-labeled Pro5® Pentamers to visualize a clear population of antigen-specific cells to a similar frequency.

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Bead Isolation Protocol (Figure 2)

Materials required Wash buffer (0.1% sodium azide, 0.1% BSA in PBS), Streptavidin beads (e.g. Lodestars 2.7 Streptavidin from Polymer Labs; Dynabeads M-280 Streptavidin from Dynal Biotech).

1. For best results start with at least 1×10^7 lymphoid cells (PBMC or splenocytes).
2. Wash cells with wash buffer (WB) and resuspend in 200µl WB.
3. Add 1 test (10 µl) biotin-labeled Pentamer per 2×10^6 cells.
4. Incubate at room temperature for 10 minutes.
5. Wash the cells in WB and resuspend in 500µl WB.
6. Add an optimally titrated amount of streptavidin beads (at least 5 beads per cell is recommended).
7. Incubate on ice for 30 minutes with mixing.
8. Bring the volume in the tube up to 2 ml with WB then place in a magnetic particle separator.
9. Leave for 3-5 minutes. If desired, supernatant can be retained for flow cytometric analysis to confirm removal of antigen-specific cells.
10. Wash the fraction containing bead:cell complexes 3 times with WB and discard supernatant.
11. Isolated bead:cell complexes may be placed in cell culture, where beads should dissociate after a few days.

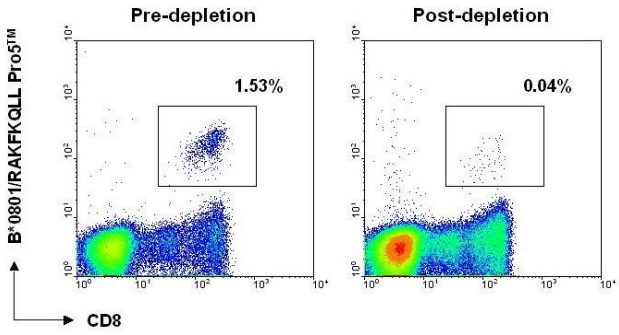

Figure 2: Antigen-specific cells were depleted from a peripheral blood suspension using a B*0801/RAKFKQLL (EBV BZLF-1)-specific biotin-labeled Pro5® Pentamer as detailed. A sample of the original cell population (pre-depletion) and supernatant following isolation (post-depletion) were incubated with anti-CD8-FITC antibody plus SA-PE to visualize antigen-specific cells. The antigen-specific population was reduced from 1.53% to 0.04%, confirming that bead isolation of EBV BZLF-1 cells was successful.

Solid-surface immobilization Protocol (Figure 3)

Materials required Phosphate-buffered saline (PBS), Streptavidin, 0.1M Sodium Bicarbonate ($NaHCO_3$), Tween-20, Bovine Serum Albumin (BSA).

1. Coat a solid surface (e.g. ELISA plate) with 100 ng / well streptavidin (100 µl / well of 96-well ELISA plate) by incubating overnight in 0.1 M NaHCO3, pH 8.2 at 4ºC.
2. Wash the surface 3 times with PBS / 0.05 % Tween-20.
3. Block with 5 % BSA / PBS (200 µl / well of 96-well ELISA plate) and incubate for 1 hour at room temperature.
4. Wash the surface 3 times with PBS / 0.05 % Tween-20.
5. Add 50ng / well biotin-labeled Pentamer (or a titration if desired) and incubate for 1hr at room temperature.
6. Wash the surface 3 times with PBS / 0.05 % Tween-20 and proceed to your desired assay.

ELISA immobilization of biotin-labeled Pro5® MHC Pentamers

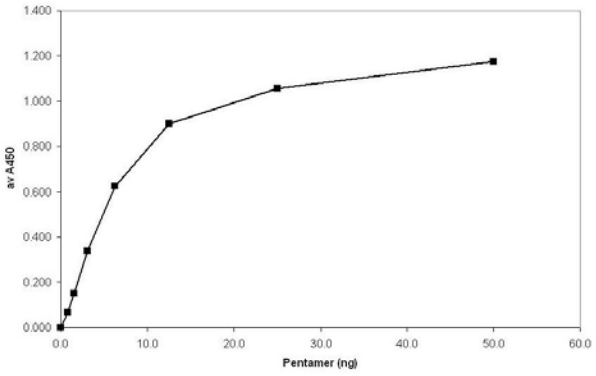

Figure 3: A 96-well ELISA plate was coated as detailed in the protocol above, using serial dilutions of an A*0201-specific biotin-labeled Pro5® Pentamer. Bound Pentamer was visualized by addition of an anti-A*0201 conformational antibody, followed by anti-mouse Ig-HRP and TMB. Plates were read at 450 nm and the plot shows Pentamer concentration against absorbance.

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 suggest you titrate all reagents to determine optimal quantities required.

Quality Control Assay Results

Appearance:	Clear, colorless solution
Protein Characterization:	Passed
MHC Conformation Immunoassay:	Passed

Released by:

(Date as per product label above)

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CERTIFICATE NO.35964

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