

**PRODUCT SHEET**

Pro5<sup>®</sup> Pentamer R-PE labeled:

- F2X-
- Fnnn-2X- (but not FN01 or F712)

**Pro5<sup>®</sup> Recombinant MHC Pentamer:**

Fluorescent-labeled Pro5<sup>®</sup> MHC Class I Pentamers are used to identify antigen-specific CD8<sup>+</sup> 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<sup>+</sup> T cells stained with Pro5<sup>®</sup> MHC Pentamers can be analyzed by flow cytometry and the frequency of antigen-specific T cells determined. Additional co-staining for intracellular cytokines (e.g. IFN $\gamma$  / IL-2) or surface markers (e.g. CD69 / CD45RO) can provide additional functional data on the antigen-specific sub-set.

**For Research Use Only. Not for use in therapeutic or diagnostic procedures.**

**Test Volume:** 10  $\mu$ l / test.

**Test Specification:** One test contains sufficient reagent to stain approximately  $1 \times 10^6$  cells. We recommend that the user titrate the reagent to determine the optimum amount to use in their specific application.

**Concentration/ Formulation:** The Pro5<sup>®</sup> Pentamer concentration is approximately 0.05 mg/ml in PBS, stabilized with 1% BSA and 0.01% sodium azide.

**Storage Condition:** 4°C. Protect from light. **Do not freeze.**

**Shelf Life:** 6 months if stored as instructed above.

**Fluorochrome:** R-phycoerythrin (R-PE) excites at 480, 565 nm and emits at 578 nm (FL-2).

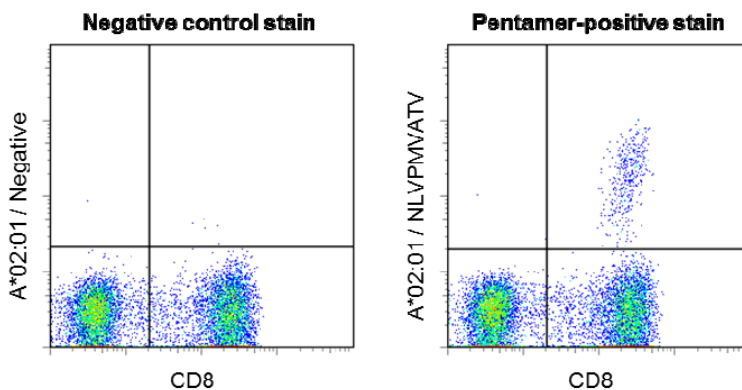
**Hazards:** This reagent is formulated in 0.01% sodium azide. Under acid conditions the toxic compound hydrazoic acid may be released. Compounds containing sodium azide should be flushed with running water while being discarded.

**Quality Control Assay Results**

**Appearance:** Clear, pale pink solution

**Protein Characterization:** Passed

**Released by:**  
(Date as per product label above)



The figure on the left shows a cell sample stained with HLA-A\*02:01 Negative Control Pentamer (Code FN01). The figure on the right shows CD8<sup>+</sup> Pentamer-positive T cells, identified in the upper right quadrant. Non-specific staining was eliminated from the plot by gating on CD19<sup>-</sup> cells before plotting CD8 vs. Pro5 MHC Pentamer.

### **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 (optional<sup>†</sup>).

1. **Centrifuge Pro5<sup>®</sup> MHC Pentamer in a chilled microcentrifuge at 14,000 ×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 taking tests for staining.
2. **Allocate 1-2 × 10<sup>6</sup> lymphoid cells (PBMCs or splenocytes) per staining condition.** Allocate only 2-5 × 10<sup>5</sup> 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 fluorescent-labeled Pro5<sup>®</sup> MHC Pentamer to the cells and mix well.**
5. **Incubate at room temperature (22°C) for 10 minutes, shielded from light.**
6. **Wash the cells with 2 ml Wash Buffer and resuspend them in the residual volume.**
7. **Add anti-CD8 and anti-CD19 antibodies to the cells and mix well.** <sup>†</sup>Use of the anti-CD19 antibody is recommended 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.

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.

### **Protocol Optimization:**

The following guidelines will help you optimize your protocol for the best possible results:

**Setting the live lymphocyte gate** It is important to ensure that the forward-scatter (FSC) and side-scatter (SSC) gates are set correctly on the cell population of interest. This is to ensure that dead cells, cell aggregates and cell debris are excluded from the fluorescence data.

**Titrating the Pro5<sup>®</sup> MHC Pentamer** Carry out a range of doubling dilutions from 1 test per 1 × 10<sup>6</sup> cells down to 1/16 test per 1 × 10<sup>6</sup> cells, in order to determine the optimum amount of Pentamer reagent to use in your specific application.

**Anti-CD8 antibody** Investigate the effect of selecting different antibody clones or titrating the anti-CD8 antibody.

**Temperature** The temperature at which cells are stained can affect signal considerably. Varying time and temperature of incubation is necessary to determine optimal signal to noise ratio depending upon the MHC/peptide combination and T cell receptor. We recommend incubation at room temperature (22°C) in the first instance, however incubating at 4°C or 37°C may be beneficial to reduce background. The higher the incubation temperature, the shorter the incubation time required.

**Positive control** Pro5<sup>®</sup> MHC Pentamers should be tested against a specific T cell line (or clone). Be sure to use T cells that have not been recently stimulated as this has been shown to cause down-regulation of T cell receptors. If a cell line is not available, use PBMCs from a known positive donor - the frequency of positive cells will be much lower and therefore sufficient events must be collected to ensure a clear result.

**Negative Control** To control for non-specific staining it is also useful to stain T cells with the HLA-A\*02:01 Negative Control Pentamer (Code FN01). Alternatively, staining T cells from unexposed individuals may be used when detecting T cell responses to a specific antigen.