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PRODUCT SHEET

Pro5® Pentamer Alphalux™ 488 Labeled:

- F11X-
- Fnnn-11X- (but not FN01 or F712)

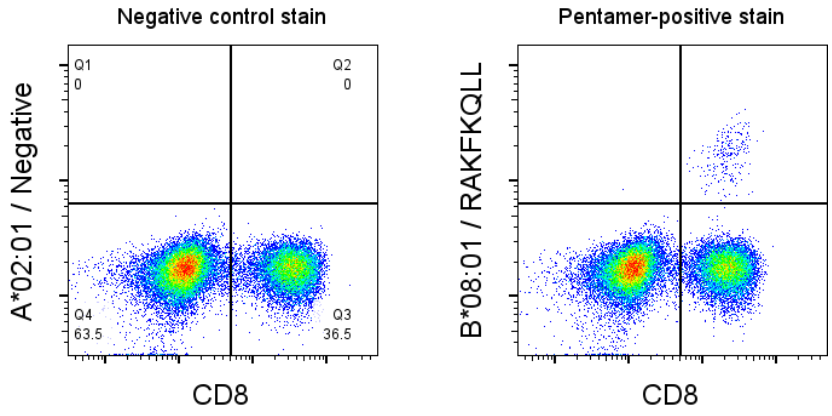
Pro5® Recombinant MHC Pentamer:	Fluorescent-labeled Pro5® MHC Class I Pentamers are used to identify antigen-specific $CD8^+$ T lymphocytes. Multimeric MHC-peptide complexes bind to T cell receptors (TCRs) of a particular specificity (as determined by the MHC allele and peptide combination). $CD8^+$ T cells stained with Pro5® 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. IFNγ / IL-2) or surface markers (e.g. CD69 / CD45RO) can provide additional functional data on the antigen-specific subset. **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 x 10^6 cells. We recommend that the user titrate the reagent to determine the optimum amount to use in their specific application.
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. Protect from light. **Do not freeze**.
Shelf Life:	6 months if stored as instructed above.
Fluorochrome:	Alphalux™ 488 excites at 493 nm; emits at 518 nm (FL-1)
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 green 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 $CD8^+$ Pentamer-positive T cells, identified in the upper right quadrant. Non-specific staining was eliminated from the plot by gating on CD19- cells before plotting CD8 vs. Pro5® MHC Pentamer.*

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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†).

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. 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 × 10^6 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 fluorescent-labeled Pro5® 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.** †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® MHC Pentamer Carry out a range of doubling dilutions from 1 test per 1 × 10^6 cells down to 1/16 test per 1 × 10^6 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® 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.

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