

PRODUCT SHEET

Pro5[®] MHC Pentamer

A*02:01 Negative Control:

- FN01-0X- ▪ FN01-1X-
- FN01-2X- ▪ FN01-4X-
- FN01-81X- ▪ FN01-82X-
- FN01-84X-

*for FN01-8nX, also require PS_A003-3

Pro5[®] Recombinant MHC Pentamer: Pro5[®] MHC Class I Pentamers are used to identify antigen-specific CD8⁺ T lymphocytes. HLA-A*02:01 negative control Pro5[®] Pentamers can be used to assess non-specific binding in flow cytometric analysis. The product consists of multimeric HLA-peptide complexes, assembled with an irrelevant peptide that is known to have no T cell response.

The HLA-A*02:01 Negative Control Pentamer is particularly recommended for use with samples where a low frequency T cell response is expected, e.g. some cancer or autoimmune epitopes. The use of a negative control reagent in conjunction with experimental Pentamer epitopes will allow low frequency positive populations to be accurately quantified.

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

Test volume: 10 µl / test for biotin- or fluorescent-labeled Pro5[®] MHC Pentamers
2 µl / test for unlabeled Pro5[®] MHC Pentamers

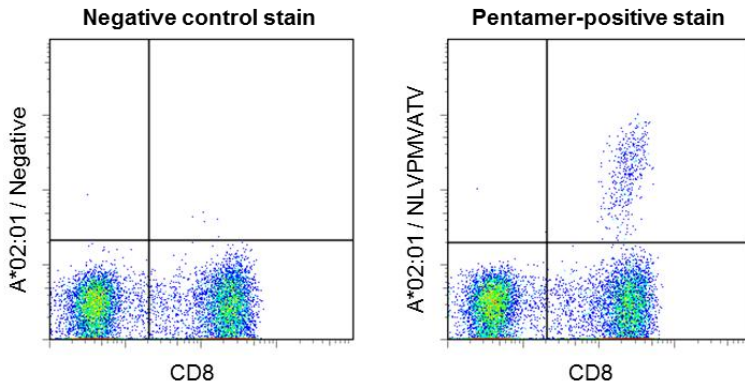
Test specification: One test contains sufficient reagent to stain approximately 1 × 10⁶ cells. We recommend that the user titrate the reagent to determine the optimum amount to use in their specific application.

Concentration/ Formulation: Biotin- or fluorescent-labeled Pro5[®] MHC Pentamer concentration is approx. 0.05 mg/ml
Unlabeled Pro5[®] MHC Pentamer concentration is approx. 0.5 mg/ml
All products are provided in PBS, stabilized with 1% BSA and 0.025% sodium azide.

Storage Condition: Fluorescent-labeled Pro5[®] MHC Pentamers may be stored at 4°C for 6 months. **Protect from light and do not freeze.**
Biotin-labeled and unlabeled Pro5[®] MHC Pentamers may be stored at 4°C for 3 months or -80°C for at least 12 months. **Avoid freeze-thaw cycles.**

Label Options: R-phycoerythrin (R-PE) excites at 480, 565 nm and emits at 578 nm (FL-2)
Allophycocyanin (APC) excites at 650 nm and emits at 660 nm (FL-4)
Biotin-labeled for use in combination with fluorescent-labeled streptavidin
Unlabeled for use in combination with Pro5[®] Fluorotag

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.



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.

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 × 10⁶ lymphoid cells (PBMCs or splenocytes) per staining condition.** Allocate only 2-5 × 10⁵ 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 of 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 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.
 For biotin-labeled Pro5 MHC Pentamers: **Include fluorescent-labeled streptavidin with the antibody mixture.**
 For unlabeled Pro5 MHC Pentamers: **Include 8 µl Pro5[®] Fluorotag with the antibody mixture.**
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

For further tips on protocol optimization refer to <http://www.proimmune.com/support-protocol-optimization> or download the Pro5[®] MHC Pentamer Handbook which contains useful protocols and advice on how to achieve the best possible staining for your samples (<https://www.proimmune.com/pro5-pentamer-handbook>).