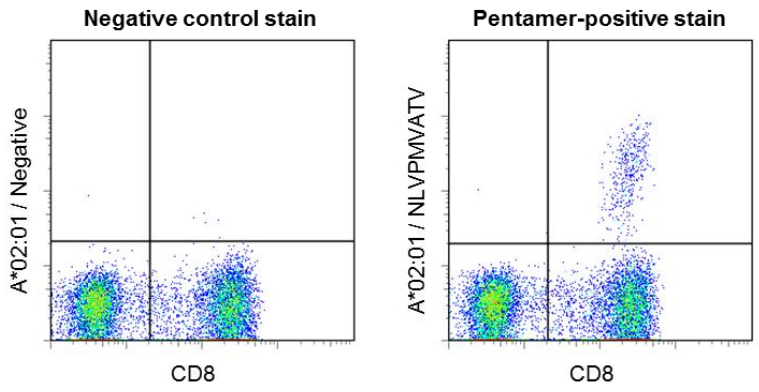
PROIMMUNE
www.proimmune.com

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 1×10^6 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.*

ProImmune Limited · The Magdalen Centre · The Oxford Science Park · Robert Robinson Avenue · Oxford OX4 4GA · United Kingdom
Tel: +44 (0)870 042 7279 · Fax: + 44 (0)870 712 0588 · Email: enquiries@proimmune.com · Web: www.proimmune.com
Registered in England No.: 387 3386 · VAT Registration No.: GB 749 9053 87

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 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).

ProImmune Limited · The Magdalen Centre · The Oxford Science Park · Robert Robinson Avenue · Oxford OX4 4GA · United Kingdom
Tel: +44 (0)870 042 7279 · Fax: + 44 (0)870 712 0588 · Email: enquiries@proimmune.com · Web: www.proimmune.com
Registered in England No.: 387 3386 · VAT Registration No.: GB 749 9053 87