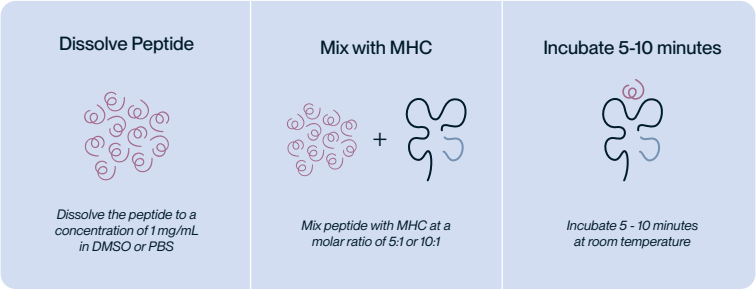
TCR Staining using PE-Labeled prMHC Tetramer

What is a peptide-ready MHC (prMHC)?

prMHCs are stabilized peptide-free MHC monomers and tetramers. They can be loaded with custom peptides in-house using our quick and simple loading protocol. prMHCs exhibit comparable activity to single chain trimer MHCs and do not use UV or peptide-exchange technology. They are suitable for generating custom MHC monomers and tetramers, performing high-throughput peptide screening, or TCR screening.

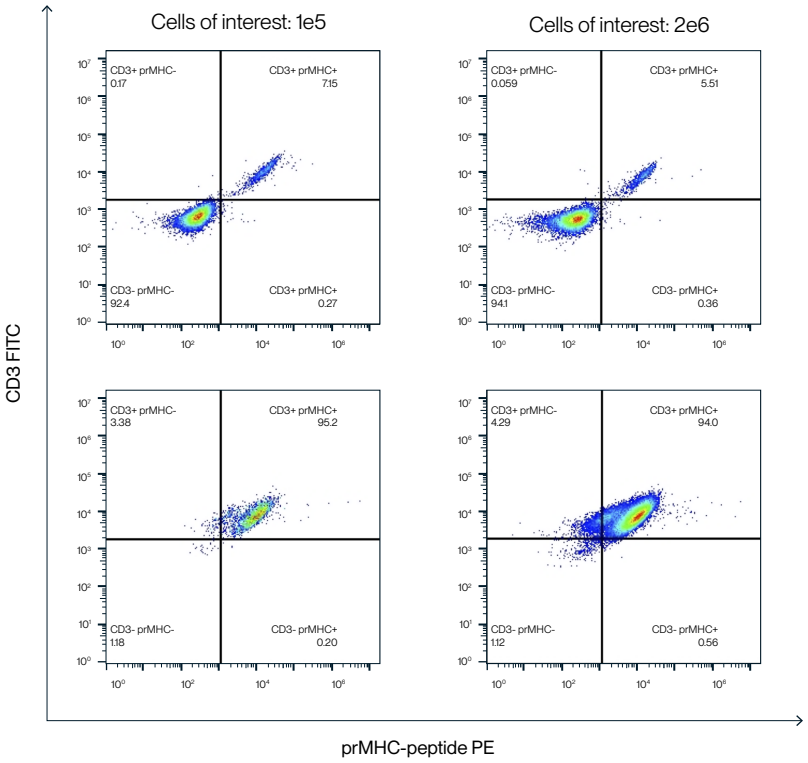


Experiment Overview

A leading TCR company conducted an experiment using two TCR-expressing cell lines: (1) incomplete TCR expression and (2) complete TCR expression. The cell lines were stained using KACTUS PE-Labeled Human Peptide Ready HLA-A*02:01&B2M Tetramer, loaded with a peptide, cognate or not (sequence proprietary) to identify antigen-specific CD3+ cells. To load the prMHC, the peptide was mixed with the prMHC-PE tetramer at a 10:1 molar ratio. For staining, 1µg of prMHC-PE was used per 1e5 and 2e6 cells.

Results: Greater than 95% CD3+ prMHC+ staining with cognate peptide

Both cell populations with complete TCR expression achieved over 94% CD3+ prMHC+ staining with near-zero non-specific staining both in CD3- populations, and when using a non-cognate peptide. In contrast, competitor MHC reagents resulted in only approximately 35% staining of CD3+ cells with 5-10% non-specific staining in CD3- populations, and when using a non-cognate peptide. This demonstrates a significant improvement over the results previously achieved with another leading MHC peptide exchange technology. Furthermore, the company performed titration experiments to successfully reduce the amount of prMHC-peptide used per stain to 1µg and to increase the number of cells to 2e6.



Cell line 1

Incomplete TCR expression

Less than 10% of cells are CD3+ prMHC+, indicating low TCR expression. The majority of cells are CD3- prMHC-, with near-zero non-specific staining.

Cell line 2

Complete TCR expression

Over 94% of cells are CD3+ prMHC+, demonstrating high TCR expression. Near-zero non-specific staining is indicated by the low percentages in the CD3- populations.