Enhanced Assay Reliability: Advanced Negative Control Dextramer® Reagents in Antigen-Specific T Cell Detection

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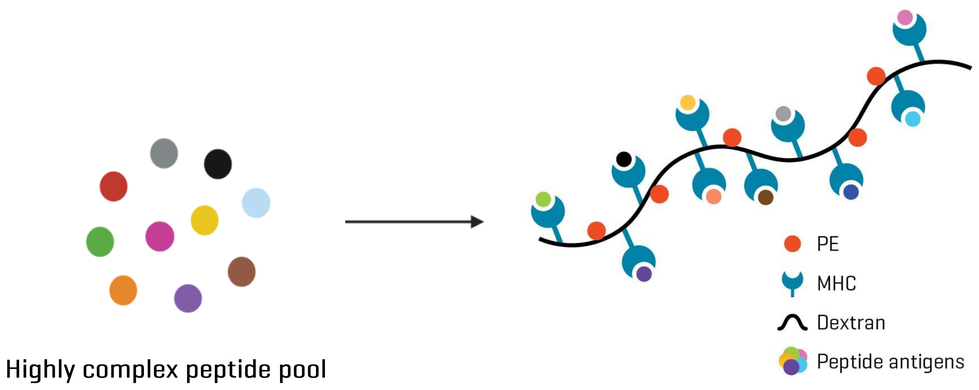
Introduction

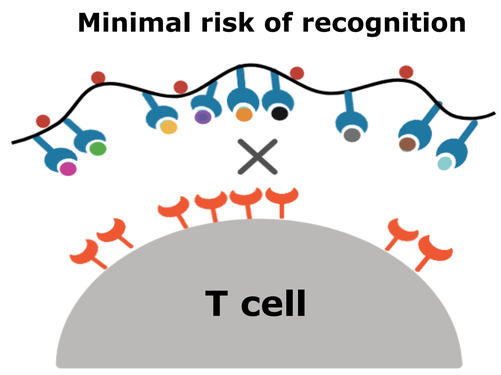
- MHC Dextramer® reagents and other peptide-MHC (pMHC) multimers are the standard tools used for analyzing antigen-specific T cells.
- MHC Dextramer® bind with strong avidity to antigen-specific T cells by engaging in multiple simultaneous interactions between the pMHCs on the Dextramer® and the cognate T cell receptors (TCRs) at the surface of T cells.
- The strong avidity effect of multimers significantly enhances monomeric affinities, making short interactions viable for cell surface staining. However, this strong avidity can also magnify weaker general protein-protein and other interactions, not directly related to antigen recognition.
- Thus, readily available and reliable negative control reagents are a necessity for establishing the threshold between background and antigen-specific staining, thereby improving the identification of positive cells, even for low-affinity pMHC-TCR responses.
- Here, we introduce the new Peptide Pool Negative Control Dextramer® reagents that due to an innovative design are unable to bind to T cells by antigen-specific TCR engagement.

Generation of Peptide Pool Negative Control Dextramer®



Highly complex peptide pool

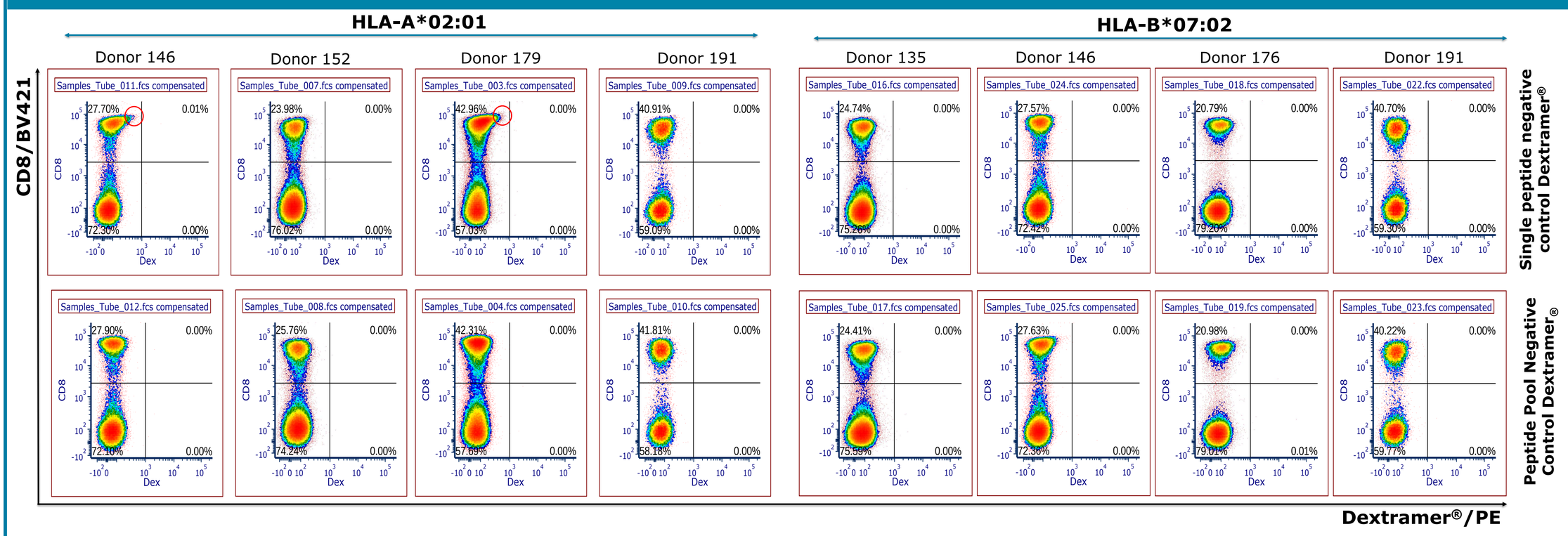
Each Negative Control Dextramer® is decorated with pMHC monomers that all display a different peptide.



The diversity of pMHC complexes on the Dextramer® prevents binding by antigen-specific TCR engagement. Residual staining therefore is a result of allele-specific, but peptide-independent binding.

Peptide Pool Negative control Dextramer® reagents are produced by refolding MHC molecules with peptide pools of enormous diversity. The resulting pool of pMHC complexes is then loaded onto a fluorescently labeled Dextran backbone to produce Peptide Pool Negative Control Dextramer® reagents. Consequently each Dextramer® is decorated with MHC monomers that all present different peptides. In addition, no two Peptide Pool Negative Control Dextramer® molecules are likely to be composed of the same combination.

Performance of Peptide Pool Negative Control Dextramer®



Human PBMCs were stained with allele-matched Peptide Pool Negative Control MHC Dextramer® reagents and traditional single peptide negative controls based on MHC monomers folded with empirically derived peptides known to stain very few T cells. The Peptide Pool Negative Control Dextramer® exhibited performance on par with the single peptide negative controls. In the case of Donors 146 and 179 and HLA-A*02:01, a slightly elevated peptide-dependent background staining was observed with the single peptide MHC Dextramer® negative control that was absent with the corresponding peptide pool-based MHC Dextramer® control.

Conclusions

- Peptide Pool Negative Control Dextramer® reagents were created using peptide pools of vast diversity.
- These controls are available for HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, and HLA-B*07:02 alleles and can be tailored for any other allele, effectively addressing the challenge of insufficient negative controls for specific alleles.
- Peptide Pool Negative Control Dextramer® demonstrated equal or better performance compared to single peptide negative controls.
- These reagents ease the gating of antigen-specific T cells and establish allele-specific background thresholds in subsequent analyses.

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