TCR Discovery and Detection of Antigen-Presentation on Cells using the Dextramer® Technology

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Introduction

To successfully develop and apply T cell-based immunotherapies, the specificity and sensitivity of the selected TCR must first be validated before proceeding to clinical development. Here, the detection and quantification of antigen-presenting cells (APC) is important for **1)** stratification and selection of patients with demonstrated expression of the target antigen, **2)** confirming tissue-specific expression of the target antigen, and **3)** monitoring target expression during treatment. To supports such efforts, we have developed high avidity TCR Dextramer® reagents to allow detection of peptide presentation by APCs. This study presents a complete workflow for TCR discovery, followed by the generation and use of TCR Dextramer® as a potential analytical tool for evaluating target expression on the cell surface of APCs.

Identify Candidate TCR Sequences using dCODE Dextramer®

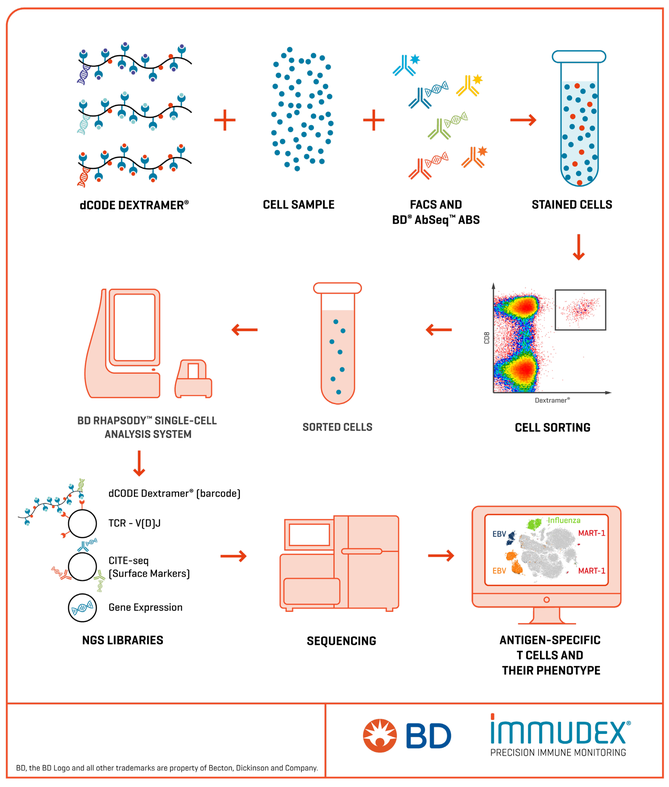
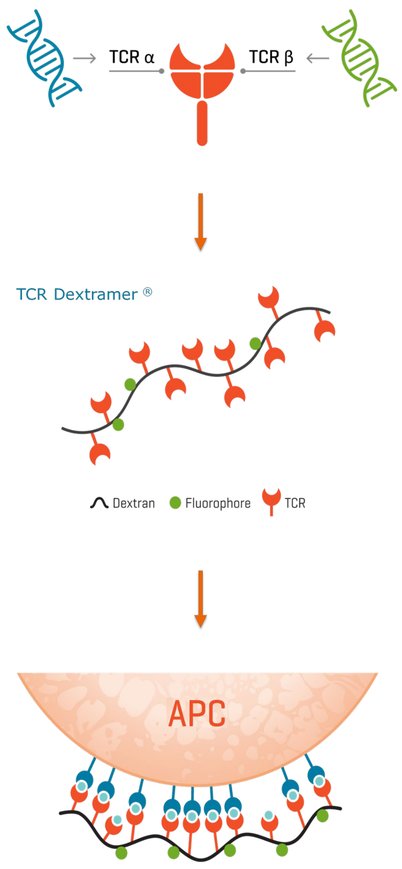


Fig. 1. Using dCODE Dextramer® Technology to identify candidate TCR sequences (with Gene Expression and Surface Markers). A smooth workflow from dCODE Dextramer® reagents via single-cell transcriptomics (10x Chromium/BD Rhapsody) to specificity-validated TCR molecules.

Optimized Manufacturing of Soluble TCR Monomers and TCR Dextramer®



Soluble TCR Monomers are produced in *E. coli*, refolded, biotinylated, and purified with an optimized platform. Rigorously QC'ed Soluble TCR Monomers are attached to a fluorescent Dextramer® backbone. Antigen-presenting cells (APC) can be stained with TCR Dextramer® reagents like conventional pMHC Dextramer® reagents on T cells.

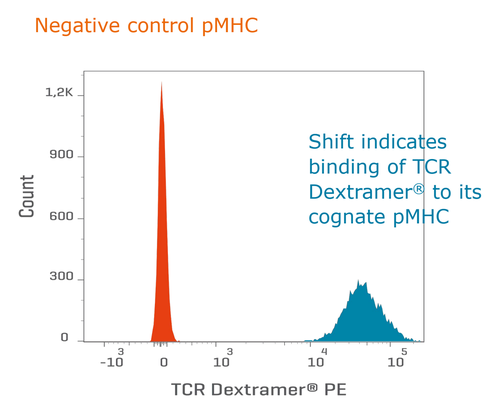


Fig. 2. Quality control of TCR Dextramer®.
TCR functionality and specificity for the target pMHC is confirmed in an artificial cell system by flow cytometry.

Generated TCR Dextramer®

TCRs from Conventional T cells

TCR ID	TCR specificity		K_d
	Allele	Antigen peptide	
A	HLA-A*02:01	SLLMWITQV	48 pM
B	HLA-A*02:01	SLLMWITQV	32 µM

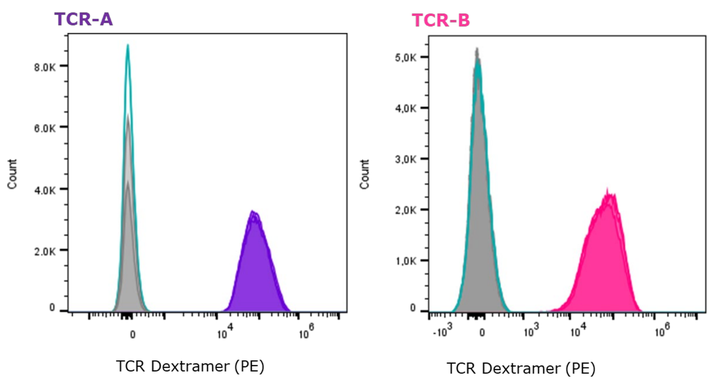
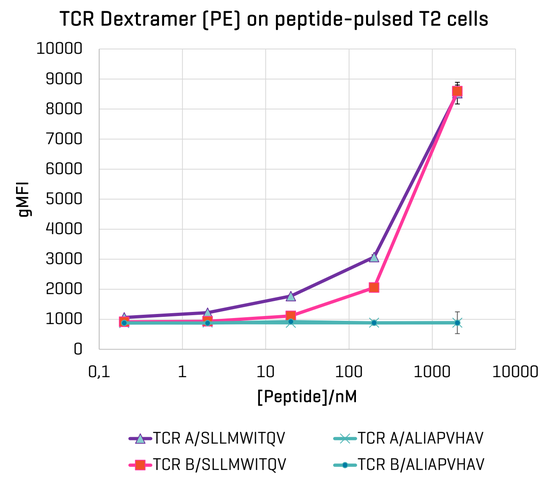


Fig. 3. Recombinant TCRs from conventional T cells with different affinities, binds specifically to their targets.

TCR-A and **TCR-B** were evaluated in a bead-based assay for binding to their target. Both TCRs, bind efficiently to HLA-A*02:01/SLLMWITQV conjugated beads (Purple and Pink peaks), but not to control peptide ALIAPVHAV (gray pic) and not to unrelated HLA-E*01:03/RLPAKAPLL (Cyan pic).



Staining of peptide-pulsed cells using TCR Dextramer®

Peptide-pulsed T2 cells are stained with TCR Dextramer® reagents based on high and low affinity TCRs. The mean fluorescence intensity of the relevant cell population is measured using flow cytometry.

Fig. 4. TCR affinity impacts TCR Dextramer® binding.
Peptide titration ranging from 0.1 nM to 10000 nM on the cells using either target peptide or control peptide.
Target peptide: SLLMWITQV, Kd (HLA-A2) = 5.6 nM; control peptide: ALIAPVHAV, Kd (HLA-A2) = 7.0 nM.

TCRs from Non-conventional T cells

TCR ID	TCR specificity	
	Allele	Antigen
C	MR1	5-OP-RU
D	HLA-E*01:03	RLPAKAPLL

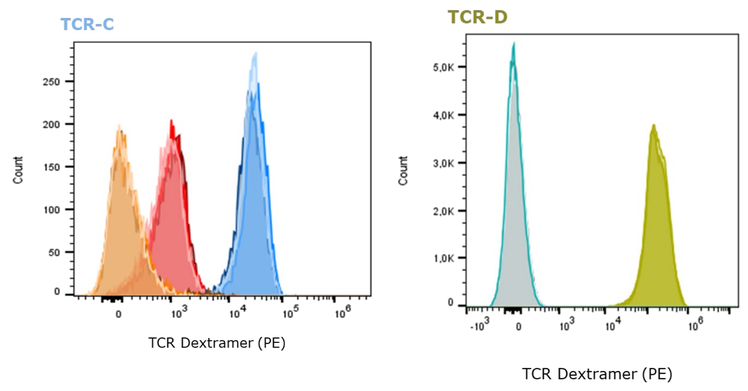


Fig. 5. Recombinant TCR proteins from non-conventional T cells binds specifically to their targets, in a bead-based assay.

Left: A MAIT TCR (**TCR-C**) identified from MAIT TCR sequences were evaluated for binding to their target, MR1/5-OP-RU. The MAIT derived TCR, bind efficiently to MR1/5-OP-RU conjugated beads (blue pic), but not to control HLA-A*02:01 (orange pic) and not to MR1/6-FP conjugated beads (red pic).

Right: HLA-E*01:03/RLPAKAPLL specific **TCR-D** can bind specifically to its target conjugated beads (green pic), but not to control HLA-A*02:01 (gray and Cyan pics).

Conclusions

- pMHC-specific TCR sequences can be identified using dCODE Dextramer® reagents in a simple workflow.
- TCR functionality can be verified by making Soluble TCR Monomers and evaluate pMHC recognition in an artificial cell system.
- Both classical and non-classical TCR proteins have been developed using Immudex TCR platform.
- TCR Dextramer® reagents can be used to detect peptide presentation at the surface of antigen-presenting cells, here shown for peptide pulsed T2 cells. Peptide presentation is detectable ≥2 nM peptide-pulsing concentration on T2 cells.
- TCR Dextramer® could be a useful tool to develop novel techniques for the detection of antigen presenting cells.

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