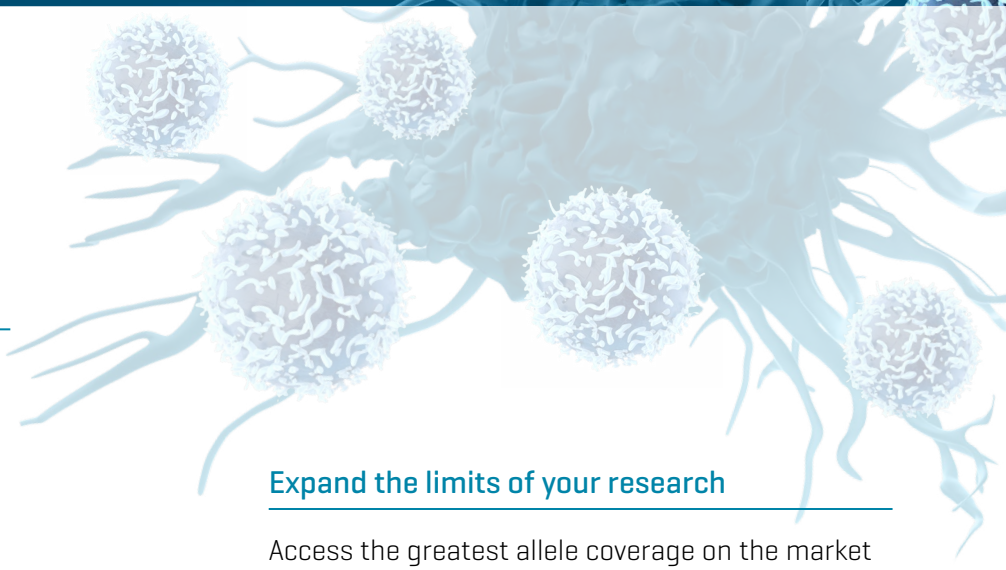
IMMUDEX®
PRECISION IMMUNE MONITORING

Advancing TCR Discovery

T-cell receptors (TCRs) allow T cells to recognize and respond to a vast array of threats, ranging from viruses to malignant cells. The interaction between the TCR and antigen thus forms a molecular switch that defines immune activation. Hence, accurate and rapid TCR discovery is critical, not just for the development of T cell-based therapies, but also for understanding the T-cell response and the effects of a given therapy.

V(D)J sequencing enables the diversity of TCR receptors to be uncovered in bulk. However, linking antigen specificity to the cognate TCR sequence, and data accuracy, are among the main challenges when studying the TCR repertoire. By combining single-cell multi-omics with the precision of the Dextramer® technology, dCODE Dextramer® reagents enable researchers to connect the antigen with the α, β, and/or paired αβ TCR sequences down to the single-cell level for advanced TCR discovery.



Cutting-Edge Dextramer® Reagents for TCR Discovery

Get the full picture of the T-cell response

The antigen-specific immune response never comprises just one specificity or affinity, but many. Dextramer® reagents enable you to cover the whole spectrum of the T-cell response, even low-affinity interactions.

Ensure consistent, reproducible, and comparable results

The high quality of Dextramer® reagents ensures reproducible results across different methods and alignment between collaborators, for example in multicentre trials.

Expand the limits of your research

Access the greatest allele coverage on the market and investigate the cellular immune response beyond T cells and using different platforms.

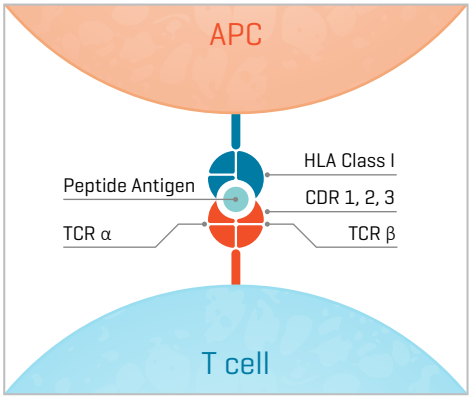
Secure flexibility in your experiments

Dextramer® reagents are optimized for use across different platforms, allowing you to continue directly from in-situ, to flow cytometry, and move onto NGS or single-cell multi-omics.

dCODE® – a Method to Investigate TCR Sequences of Therapeutic Value

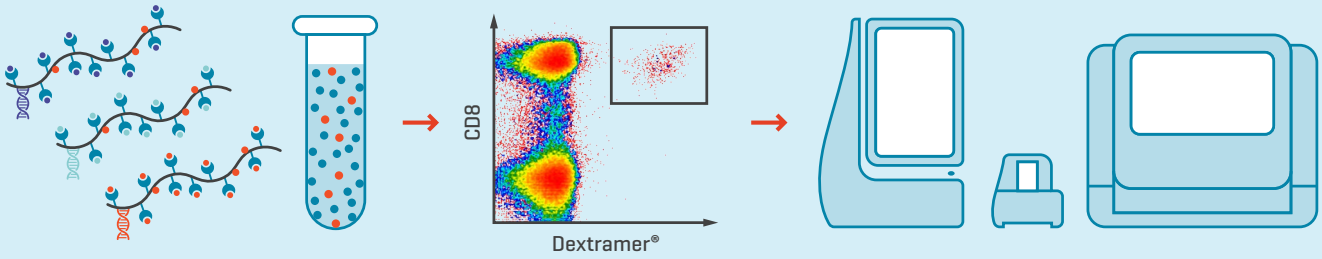
Antigen exposure shapes our unique repertoire of TCRs that are a critical component of immunological memory[1]. Generating more and higher quality TCR data may allow the identification of new biomarkers for disease and advance T-cell therapies through the identification and ex vivo monitoring of clones with therapeutic value[2,3]. However, only a fraction of the TCR repertoire has today been sampled due to limitations in the sensitivity of some sequencing methods, making it difficult to screen and select TCRs for therapeutic use[2].

The specificity of the TCR toward its cognate pMHC target is carried by the CDR3 of both α- and β-chains[4,5]. Even so, most TCR data-generating studies have focused on the β-chain alone. dCODE Dextramer® enables V(D)J sequencing of the α, β, and/or paired αβ chains of antigen-specific T cells with unprecedented precision at which even rare TCRs can be estimated[6,7].



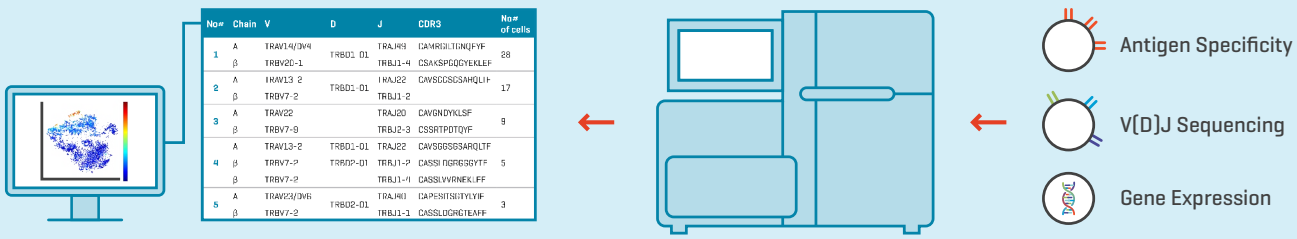
TCR Discovery with dCODE Dextramer® Workflow

The TCR discovery process with dCODE Dextramer® reagents starts with sample staining. Independent of your sample type, cells are first stained with dCODE Dextramer®, followed by antibody staining if you wish to look for cell-surface markers. The fluorophore on dCODE Dextramer® enables sorting of antigen-specific T cells by FACS. Once sorted, cells of interest are partitioned into single-cell reactions on the BD Rhapsody™ or 10x Chromium system. Cells are then lysed to ensure release of mRNA, and cDNA libraries are prepared for antigen specificity, V(D)J sequencing, and potentially surface marker- and gene expression. Once sequenced, data is analyzed to obtain information on TCR sequences.



Cell Staining and Isolation of Candidate T Cells from Patients Responding to Immunotherapy or Disease

Partitioning of Candidate T Cells into Single-Cell Reactions on BD Rhapsody™ or 10x Chromium



Identification of TCR and Target Pairs from Candidate Antigen-Specific T Cells

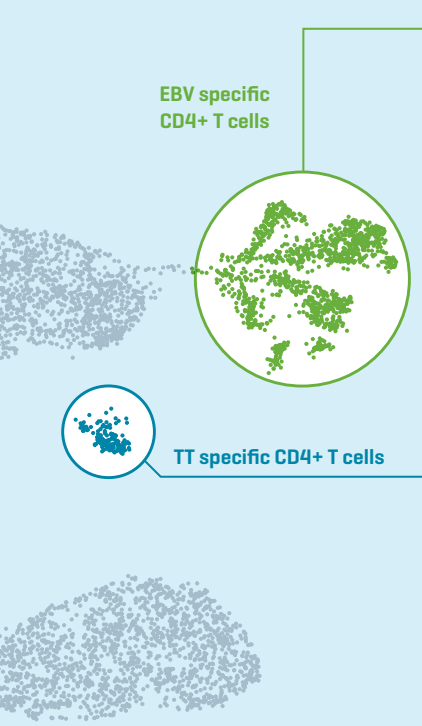
Sequencing

cDNA Library Formation

Discover the Link between the TCR and the Antigen

Identification of TCRs and target antigens with therapeutic relevance can be improved by considering low abundance or lower affinity disease-reactive TCRs, cell phenotype, and their heterogeneous nature, in addition to abundant TCRs[8,9]. Methods that enable bulk analysis of immune cells, like RNA-seq, are often dominated by the most abundant cells and lack the resolution for rare and lower affinity cells that may be of therapeutic importance.

The stability and high number of MHC monomers on dCODE Dextramer® technology ensures a highly sensitive and efficient detection of antigen-specific T cells, regardless of their affinity and abundance[10,11]. Combined with surface marker and gene expression data, the TCR repertoire sequencing further allows for the quantitative tracking of high- and low-affinity T-cell clones as they go through expansion and contraction phases of a disease response[7]. Thereby, TCR sequencing can help track changes in the T-cell repertoire during disease, response to therapy, cross reactivity, but also to select candidate TCRs for immunotherapy[7,12,13]. Once the TCR clones of therapeutic value have been identified, MHC Dextramer® reagents can be used for enrichment of TCR specific clones and custom TCR Dextramer® reagents can be applied to validate antigen presentation on target cells[14].



EBV specific CD4+ T cells

No#	Chain	V	D	J	CDR3	No# of cells
1	A	TRAV38/DV8	TRBD2-01	TRAJ27	CALYNTNAGKSTF	492
	β	TRBV29-1		TRBJ2-1	CSVDRGVGYEQEF	
2	A	TRAV38-2/DV8	TRBD1-01	TRAJ27	CAVYNTNAGKSTF	50
	β	TRBV29-1		TRBJ2-3	CSVESAGAGDTQYF	
3	A	TRAV38-2/DV8	TRBD2-01	TRAJ27	CALYNTNAGKSTF	36
	β	TRBV29-1	TRBD1-01	TRBJ2-1	CSVDRGVGYEQEF	
	β	TRB29-1		TRBJ2-3	CSVESAGAGDTQYF	
4	A	TRAV38-2/DV8	TRBD2-01	TRAJ27	CALYNTNAGKSTF	17
	β	TRBV29-1		TRBJ2-7	CSAEEAGSGDEQYF	
5	A	TRAV29/DV5	TRBD1-01	TRAJ30	CAALRDDKIIF	9
	β	TRBV7-8		TRBJ2-1	CASSSRGRLSIEQEF	

TT specific CD4+ T cells

No#	Chain	V	D	J	CDR3	No# of cells
1	A	TRAV14/DV4	TRBD1-01	TRAJ49	CAMRGILTGNQFYF	28
	β	TRBV20-1		TRBJ1-4	CSAKSPGQGYEKLEF	
2	A	TRAV13-2	TRBD1-01	TRAJ22	CAVSGGSGSARQLTF	17
	β	TRBV7-2		TRBJ1-2		
3	A	TRAV22		TRAJ20	CAVGNDYKLSF	9
	β	TRBV7-9		TRBJ2-3	CSSRTPDTQYF	
4	A	TRAV13-2	TRBD1-01	TRAJ22	CAVSGGSGSARQLTF	5
	β	TRBV7-2	TRBD2-01	TRBJ1-2	CASSLDGRGGGYTF	
	β	TRBV7-2		TRBJ1-4	CASSLVVRNEKLFF	
5	A	TRAV23/DV6	TRBD2-01	TRAJ40	CAPESITSGTYLYIF	3
	β	TRBV7-2		TRBJ1-1	CASSLDGRGTEAFF	

What antigen-specific TCRs emerge after stimuli with immunogenic antigens?

What TCR pairs to selected antigens?

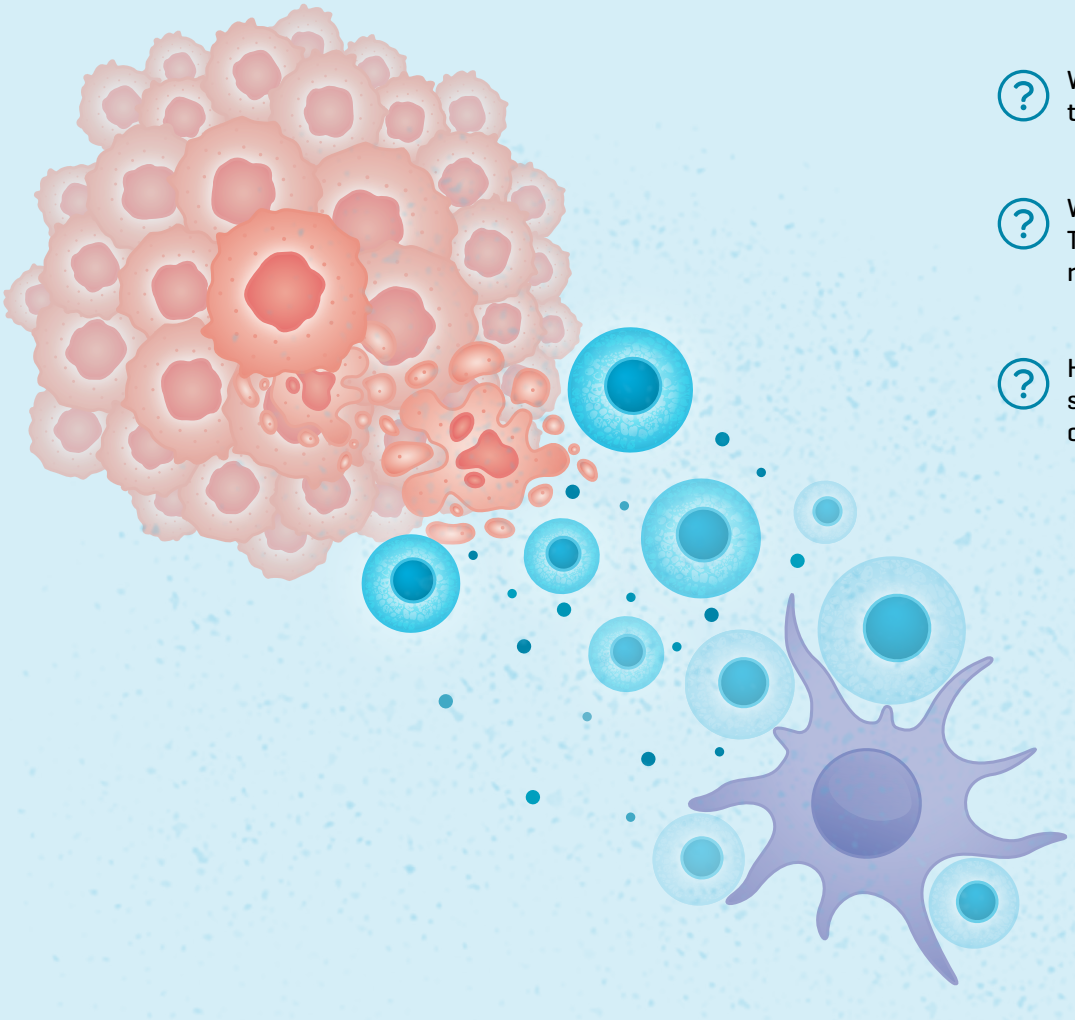
Which antigens most potently enhance the TCR selection?

TCR Discovery with dCODE Dextramer® Technology. Single-cell multi-omic analysis with dCODE Dextramer® enables extensive characterization of antigen-specific CD8+ or CD4+ T cells by adding information on gene expression, surface protein expression, and TCR sequence by V(D)J sequencing. dCODE Dextramer® reagents are barcoded with DNA oligonucleotides, providing almost infinite characterization possibilities. Immune receptor mapping provides cell type, cell state, clonotype, and antigen-binding information from the same single cells. In the end, all this may help you discover TCRs with therapeutic value, and track and deeply characterize T cells for accelerated T-cell therapy[13,15,16,17]. dCODE Dextramer® is also available as U-Load dCODE Dextramer®, which you can load with peptide-receptive MHC monomers, enabling flexible investigation of TCR specificities of your choice. Data shows TCR sequences of Tetanus Toxoid (TT) or EBV-specific CD4+ T cells. Details of the experiment can be found here.

Discover TCRs with Greater Therapeutic Value

Cross-reactivity is the capacity of a TCR to recognize more than one peptide-MHC molecule and is one of the major challenges in the clinical development of TCR-based therapies[18]. Extensive characterization of the interaction between the TCR and antigen may provide the foundation to predict the risk of side effects of individual TCRs before applied as a therapeutic measure[8,9].

Peptide-receptive U-Load dCODE Dextramer® reagents may address toxicity related issues and enable identification and extensive characterization of TCRs that are responsive to, for instance, neoepitopes. Analysis of gene and surface marker expression in addition to V(D)J sequencing of antigen-specific T cells using single-cell multi-omics can further characterize the identified TCRs down to the single-cell level[6,19,20]. Additionally, dCODE Dextramer® or MHC Dextramer® reagents can be applied to efficiently monitor selected TCRs once applied as a therapeutic on its own or in combination with other drug delivery methods by single-cell multi-omics or flow cytometry, respectively[6,15,19].



- What TCR predicts the lowest toxicity?
- Which neoepitope specific TCRs elicit most efficiently response to disease?
- How can drug delivery strategies impact duration of TCR based therapies?

Deepen your Understanding of the TCRs. Dextramer® reagents can help you understand how a given epitope or cell therapy impacts the antigen-specific immune response *ex vivo*[15]. Monitoring antigen specificity can provide insights into what antigen-specific T cells are present and exert anti-cancer responses within the tumor microenvironment and which shared tumor-associated antigens induce effect T-cell responses across cancers[16]. Investigating antigen specificity may provide essential insights into drug delivery strategies' effect, including impact on the duration of adoptive cell transfer and whether the drug of choice affects tumor relapse[15]. The U-Load Dextramer® reagents can be customized and loaded with your peptide of choice in your lab to swiftly change alleles or peptides on the reagent in epitope discovery[21] or neoantigen screenings[16].

Dextramer® Reagents for the Investigation of TCRs

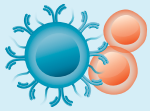
Immudex provides a range of ready-to-use, customizable, and peptide-receptive Dextramer® reagents enabling investigation, isolation, and characterization of TCRs across different platforms, including in-situ, to flow cytometry, NGS, and single-cell multi-omics. furthermore, by offering the greatest portfolio of murine and primate alleles on the multimer market, Immudex enables preclinical[6,15,16,21,22] and clinical[23,24] studies of antigen-specific T cells across animal models and patient cohorts[6,15,19].

CD8+ or CD4+ T cells

- **MHC Dextramer®** ready-to-use reagents for detection of antigen-specific CD8+ or CD4+ T cells.
- **dCODE Dextramer® (HiT)** ready-to-use reagents for bulk analysis of antigen-specific CD8+ or CD4+ T cells by NGS.
- **dCODE Dextramer® (RiO, 10x)** ready-to-use reagents for single-cell multi-omics of antigen-specific CD8+ or CD4+ T cells with information on gene expression, surface marker expression, and V(D)J sequences on the or BD Rhapsody™ 10x Genomics Chromium single-cell analysis platforms.
- **U-Load Dextramer®** reagents to be loaded with peptide receptive easYmer® or U-Load MHC II monomers of your choice in your own lab for flexible detection of antigen-specific CD8+ or CD4+ T cells.
- **U-Load dCODE Dextramer®** for flexible detection of antigen-specific CD8+ or CD4+ T cells by NGS or single-cell multi-omics. Available in HiT, RiO, or 10x format.

NKT cells

- **CD1d Dextramer®** ready-to-use or loadable reagents for reliable detection of NKT cells by flow cytometry or in-situ staining.
- **CD1d dCODE Dextramer®** for detection of NKT cells. Available in HiT, RiO, or 10x format.



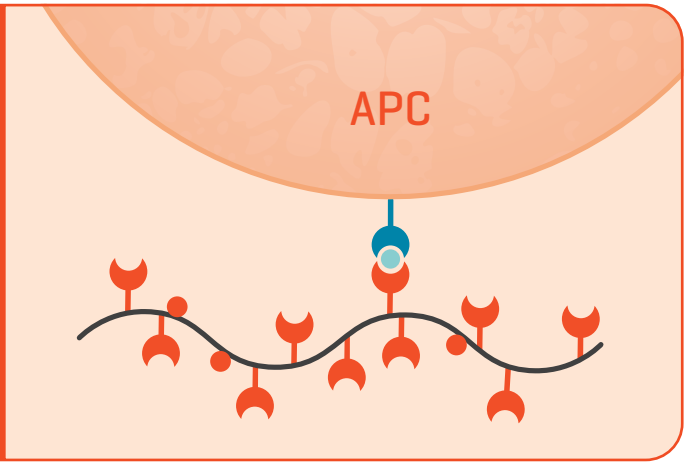
Other immune cells

- **Klickmer®** reagents for custom detection of immune cells by attachment of biotinylated molecules to the dextran backbone.
- **dCODE Klickmer®** reagents for custom detection of immune cells by NGS or single-cell multi-omics. Available in HiT, RiO, or 10x format.

• Ready-to-use • Loadable • Customizable • Flow Cytometry and In-situ Staining • NGS or Single-Cell Multi-Omics

Do You Have a Complex Research Question?

We offer **Custom Solutions and Services** tailored to your needs providing unique Dextramer® products for unique research applications. We work with you to tailor solutions and protocols, create custom high-quality reagents, and guide successful research strategies. Examples of previous Custom Solutions and Services include design of soluble TCRs or development of TCR assembled on a Dextramer® molecule.



Resources

We are dedicated to helping you get the most out of your Dextramer ® reagents by offering multiple helpful resources and support:

Resources

Easy access to our complete library of publications, application notes, webinars, protocols, and many other useful resources.

Read more

Technical Support

Let us know if you experience difficulties or have questions. Immudex will help you get the most out of your dCODE Dextramer® products.

customer@immudex.com

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