

# Simultaneous phenotype, gene expression, and TCR sequence analysis of 5-OP-RU-specific MAIT cells

**Phenotypic, transcriptomic, and TCR characterization of ligand-specific mucosal-associated invariant T (MAIT) cells from peripheral blood mononuclear cell (PBMC) samples using MR1 dCODE Dextramer<sup>®</sup> reagents and single-cell multi-omics**

Bjarke Endel Hansen, Matilda Weywadt, Kivin Jakobsen, Liselotte Brix  
Immudex ApS, Copenhagen, Denmark

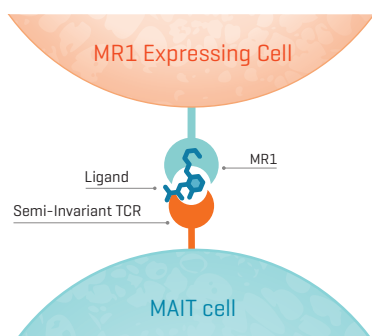
## Highlights

- We confirmed that MR1 dCODE Dextramer<sup>®</sup> reagents are a reliable and more specific tool to identify and characterize ligand-specific MAIT cell populations at single-cell resolution compared to antibodies alone.
- MR1 dCODE Dextramer<sup>®</sup> reagents proved compatible with the BD Rhapsody™ System for the multi-omic analysis of 5-OP-RU-specific MAIT cells.
- Our phenotypic, transcriptomic, and TCR sequence analysis of MAIT cells using MR1 dCODE Dextramer<sup>®</sup> reagents and the BD Rhapsody™ System corroborated published insights about the functional state, TCR diversity, and gene expression of blood 5-OP-RU-specific MAIT cells.
- We open questions about the clonotypic and functional diversity of MAIT cells in health and disease that can be addressed with MR1 dCODE Dextramer<sup>®</sup> reagents.

## Introduction

First described in 1993,<sup>1</sup> mucosal-associated invariant T (MAIT) cells are unconventional T cells activated by vitamin metabolites presented on MR1, a non-polymorphic MHC I-like molecule found on dendritic cells, monocytes, macrophages, B cells, and epithelial cells of various tissues.<sup>2</sup> Although abundant in mucosal tissues, MAIT cells are also found in the lungs, liver, joints, and blood. In healthy humans, MAIT cells constitute up to 10% of blood T cells and 45% of liver T cells.<sup>3</sup> Exposure to MR1 during intrathymic development triggers a different development path than conventional T cells.<sup>3</sup> As a result, the effector functions of MAIT cells are a fast innate-like response involving pro-inflammatory cytokine secretion and cytolytic molecule production while also supporting adaptive immunity.<sup>2,3</sup> MAIT cells can be activated through MR1-independent signaling.<sup>2</sup> Nevertheless, a defining characteristic of these cells is their semi-invariant T cell receptor (TCR) capable of recognizing MR1 ligands derived from bacterial and fungal riboflavin and folic acid synthesis. The most potent activating ligands identified to date are 5-[2-oxopropylideneamino]-6-D-ribitylaminouracil [5-OP-RU] and 5-[2-oxoethylideneamino]-6-D-ribitylaminouracil [5-OE-RU].<sup>4</sup> The folic acid derivative 6-formyl pterin [6-FP] is a non-stimulatory ligand.<sup>5</sup>

## The potential of the semi-invariant TCR of MAIT cells



MAIT cells are called invariant because their TCR consists of a semi-invariant  $\alpha$  chain associated with a limited array of  $\beta$  chains.<sup>3</sup> In humans, the predominant  $\alpha$  chain consists of TRAV1-2 with TRAJ33, TRAJ20 or TRAJ12 and the most common  $\beta$  chains include TRBV20 or TRBV6 [Table 1].<sup>3</sup> This restricted TCR variance along with the abundance and HLA-independent activation of MAIT cells make them attractive candidates for clinical translation<sup>2</sup> and several studies have examined their involvement in cancer, autoimmune disorders, wound healing, and infectious disease.<sup>2,3,6</sup> Intriguingly, the variation seen in the TCR composition of MAIT cells appears to be tissue-specific and correlates with different responses and transcriptomic programs.<sup>4,7</sup> The existence of functionally differentiated MAIT cell subpopulations is being explored<sup>7,8</sup> and raises questions about a potential repertoire of immune responses that can be used for therapeutic purposes. The answers will only come from in-depth MAIT cell characterization using high-sensitivity reagents and high-resolution methods.

Table 1: : **Characteristics of human MAIT cells**<sup>3-5,9</sup>

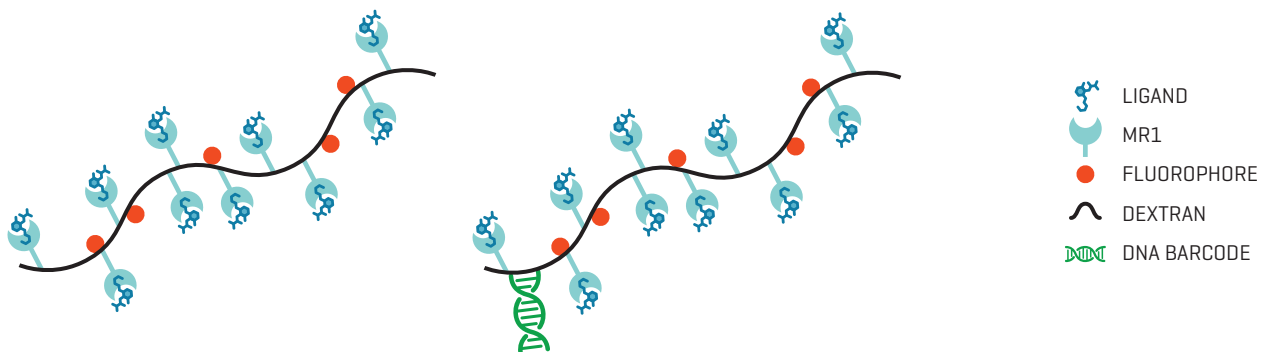
Dominant TCR $\alpha$ chains	TRAV1-2 with TRAJ33, TRAJ12, TRAJ20
Dominant TCR $\beta$ chains	TRBV6, TRBV20
Coreceptors	CD8 [70-80% circulating MAIT cells], double-negative [~15% circulating MAIT cells], CD4 [ $<5\%$ circulating MAIT cells]
Antigens (partial list)	Potent activation: 5-OP-RU, 5-OE-RU Weak activation: RL-6, 7-diMe, RL-6-Me-7-OH, diclofenac No activation, competitive inhibition: 6-FP, Ac-6-FP*
NK cell-related marker	CD161 <sup>high</sup>
Memory phenotype markers	CD45RO <sup>+</sup> , CD27 <sup>+</sup> , CCR7 <sup>-</sup> , CD95 <sup>high</sup> , CD44 <sup>high</sup> , CD62L <sup>low</sup>
Activation markers	
Steady state	CD69 <sup>+</sup> , HLA-DR <sup>+</sup> , CD25 <sup>low</sup> , CD137 <sup>int</sup>
Inflammation	CD69 <sup>+</sup> , CD25 <sup>+</sup> , HLA-DR <sup>+</sup> , CD38 <sup>+</sup> , CD137 <sup>+</sup> , CD107a <sup>+</sup>
Tissue residency markers	CD69 <sup>+</sup> , CD103 <sup>+</sup> , CLA <sup>+</sup>
Cytokine and chemokine profile	IFN- $\gamma$ , TNF, IL-2, IL-17, IL-22, GM-CSF; chemokines such as XCL1, CCL3, CCL4, and CXCL16
Integrin and chemokine receptors	CCR5 <sup>high</sup> , CCR6 <sup>high</sup> , CXCR6 <sup>high</sup> , CCR9 <sup>int/negative</sup> , among others

\* Not all MAIT cells bind 6-FP and Ac-6-FP. In this study, PBMC staining with 6-FP MR1 dCODE Dextramer<sup>®</sup> reagents produced no binding, confirming the lack of specificity for this ligand.

## MR1 dCODE Dextramer<sup>®</sup>: a detailed look at MAIT cells

With a flexible dextran backbone carrying multiple MR1-ligand complexes and fluorophores, MR1 Dextramer<sup>®</sup> reagents afford heightened avidity and boosted detection signal in the analysis of MR1-restricted cells. This molecular design captures even low-affinity and low-abundance cell populations, making MR1 Dextramer<sup>®</sup> reagents sensitive and reliable tools for MAIT cell characterization.

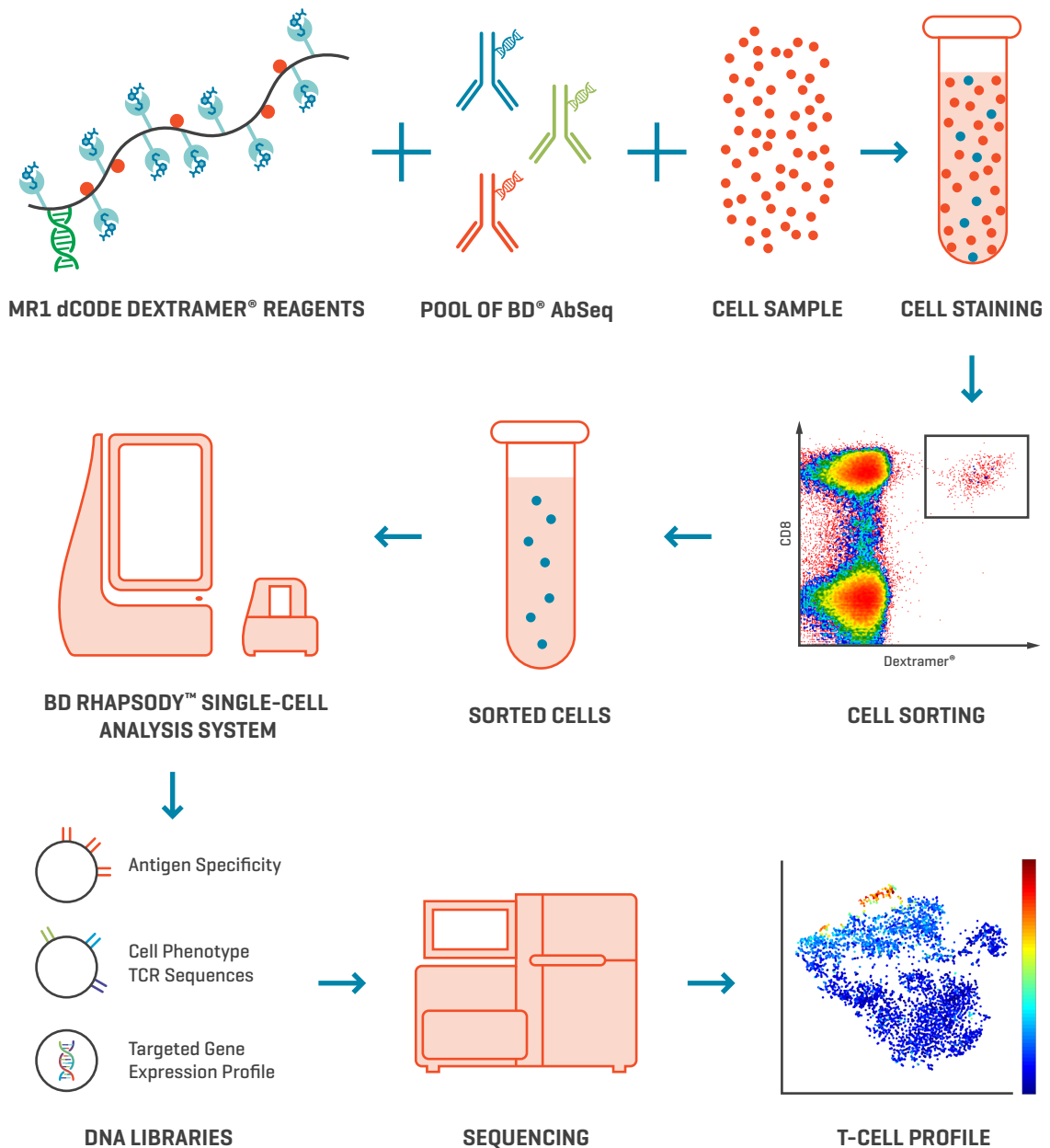
### MR1 Dextramer<sup>®</sup> and MR1 dCODE Dextramer<sup>®</sup>



**Fig. 1:** Enhances sensitivity in in-situ staining and flow cytometry, with customizable fluorophores [BV421, FITC, PE, APC, or none.] while MR1 dCODE Dextramer<sup>®</sup> includes DNA barcodes for single-cell multi-omics, revealing antigen specificity, gene/surface markers, and TCR sequencing. Together, MR1 Dextramer<sup>®</sup> technology allows progressive analysis of ligand specific MAIT cells.

Building on the Dextramer<sup>®</sup> architecture, MR1 dCODE Dextramer<sup>®</sup> [RiO] reagents include a DNA barcode that enables their identification and quantification via sequencing. These reagents are compatible with the BD Rhapsody<sup>™</sup> System, BD<sup>®</sup> AbSeq Antibody-Oligonucleotide Conjugates, and Sample Tags for multi-omic analysis of cells in the same sample and at single-cell resolution. MR1 dCODE Dextramer<sup>®</sup> reagents can be purchased preloaded with 5-OP-RU for ligand-specific MAIT cell detection and potent activation, or with 6-FP as a negative control. Here we examine the performance of 5-O-RU MR1 dCODE Dextramer<sup>®</sup> reagents in generating data on the phenotype, gene expression, and cell surface marker expression of 5-OP-RU-specific MAIT cells.

## The full immunological profiling of immune cells in a single workflow



**Fig. 2:** The full immunological profiling of immune cells in a single workflow by combining dCODE Dextramer® reagents and the BD® multi-omics platform.

### Get more details on the single-cell multi-omics workflow!

The following publication is a more detailed tour of single-cell multi-omics using the BD Rhapsody™ System and the multidimensional readout of the data it generates.

Ulbrich, J. *et al.* [2022] BD Rhapsody™ Single-Cell Analysis System Workflow: From Sample to Multimodal Single-Cell Sequencing Data. DOI: [10.1007/978-1-0716-2756-3](https://doi.org/10.1007/978-1-0716-2756-3)

## Methods

The methods and data presented here were part of a larger study using a pool of dCODE Dextramer® reagents, including spike dCODE Klickmer®, CD1d dCODE Dextramer® and MR1 dCODE Dextramer® reagents.

### dCODE Dextramer® reagents

- MR1 dCODE Dextramer® (RiO)-PE 5-OP-RU [50 tests; Cat. No. ZA80004dRG]
- dCODE Dextramer® (RiO)-PE A0201 ALIAPVHAV [50 tests; Cat. No. WB2666dRG] as a negative control

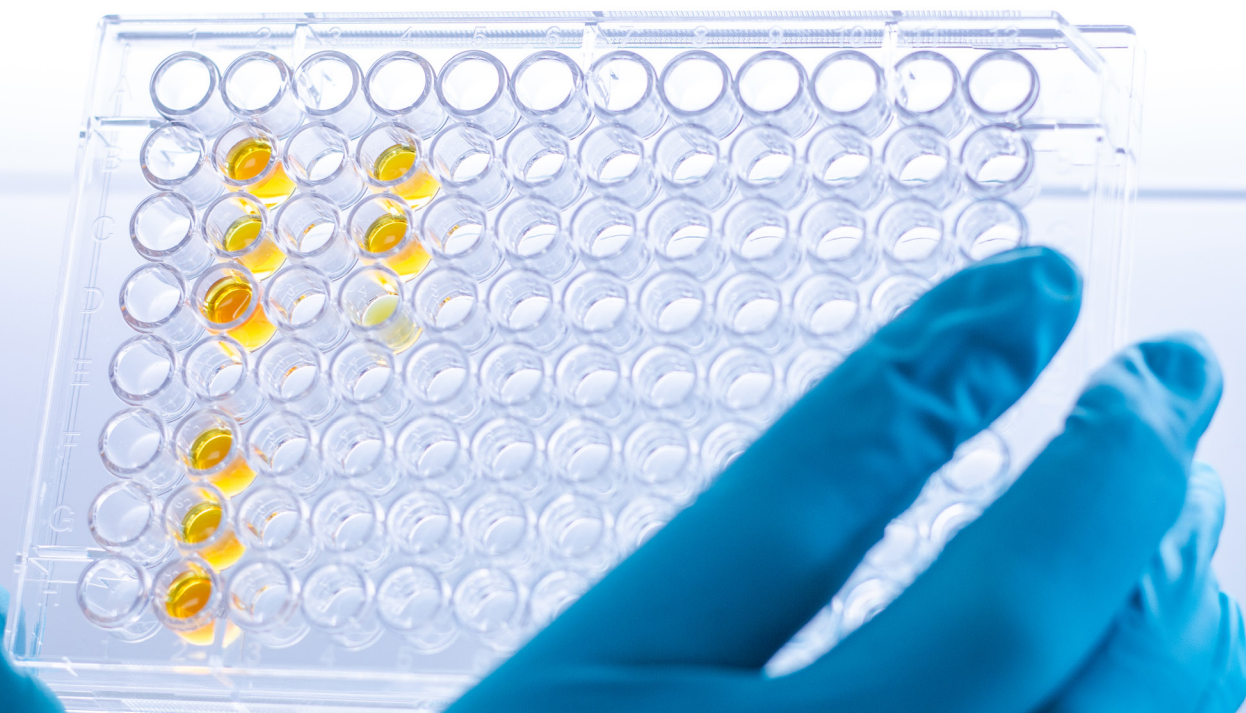
### Other reagents

- CD3-BV421; clone UCHT1 [BD® Cat. No. 562426]
- CD19-BV421; clone HIB19 [BD® Cat. No. 562440]
- Calcein-FITC [Invitrogen® Cat. No. C3100MP]
- [BD® AbSeq Immune Discovery Panel](#) [BD® Cat. No. 625970; see Appendix, Supplementary Table 1]
- SepMate™ PBMC Isolation Tubes [STEMCELL Technologies Cat. No. 85450]

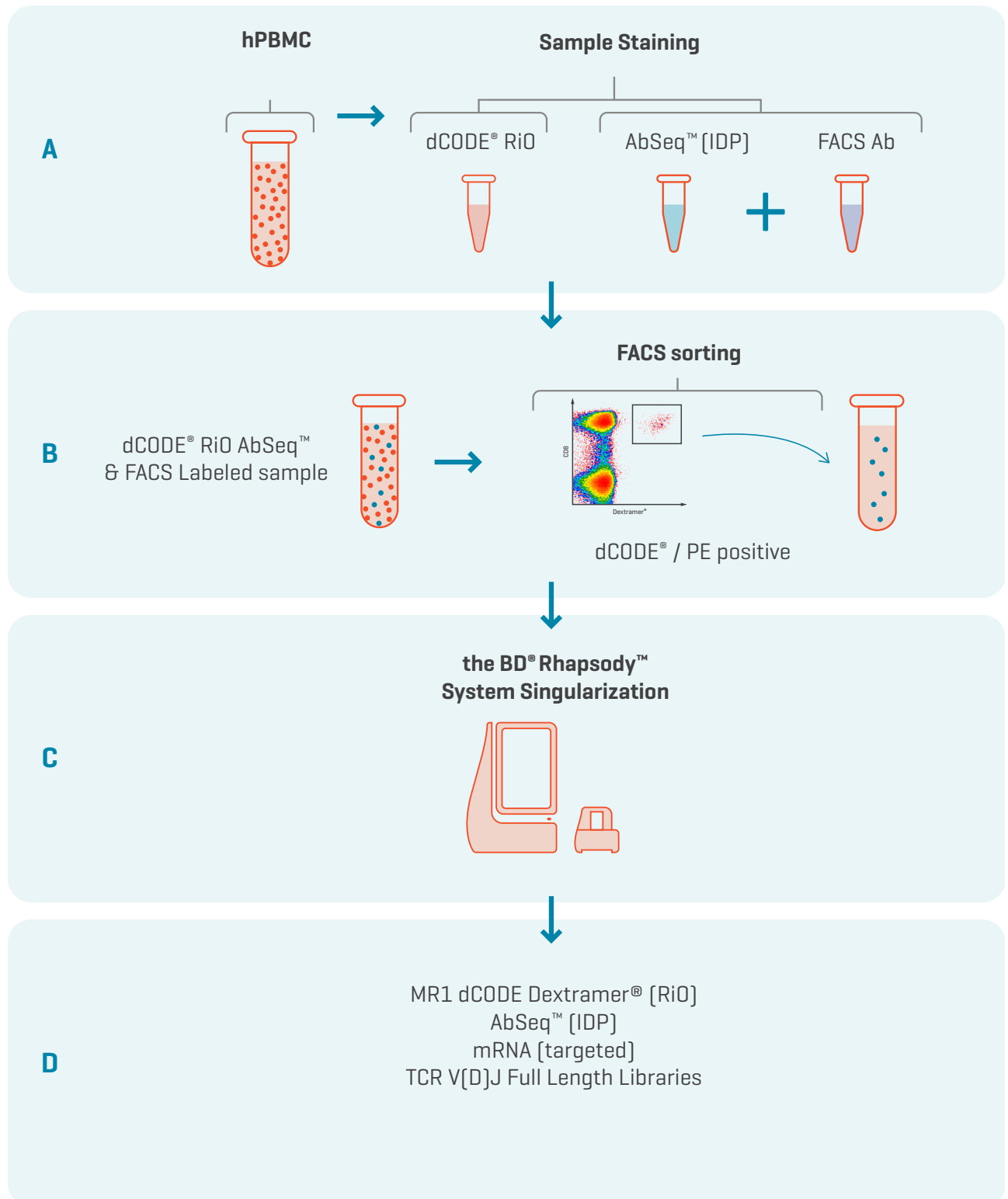
### Sample processing and cell sorting

Immediately after whole blood collection from three healthy donors into heparin tubes, we extracted peripheral blood mononuclear cells (PBMCs) using a SepMate™ PBMC Isolation Tube. The isolated PBMCs were cryopreserved for later use. Directly after thawing, the PBMCs were first stained with Sample Tag antibodies, subsequently pooled, and then stained with MR1 dCODE Dextramer®, sorting antibodies, viability dye, and conjugate antibodies of the BD® AbSeq Immune Discovery Panel (Fig. 3A). For details about the staining process, see the [dCODE Dextramer® \(RiO\) staining protocol](#).

The cells selected for singularization on the BD Rhapsody™ System [input cells] were defined as the population of 5-OP-RU MR1 dCODE Dextramer®-positive cells among CD3<sup>+</sup> or CD19<sup>+</sup> cells in the PBMC samples. Thus, the population included B cells, monocytes, T cells, and others. This sorting was done on a BD® FACS Melody™ at sort rate 293 with 100% efficiency, resulting in 437,389 sorted events (Fig. 3). Fig. 4 illustrates our gating strategy. After singularization, subsequent single-cell analyses were done on the subset of this population consisting of the ligand-specific MAIT cells defined as CD3<sup>+</sup>CD161<sup>+</sup>5-OP-RU MR1 dCODE Dextramer®-positive cells.

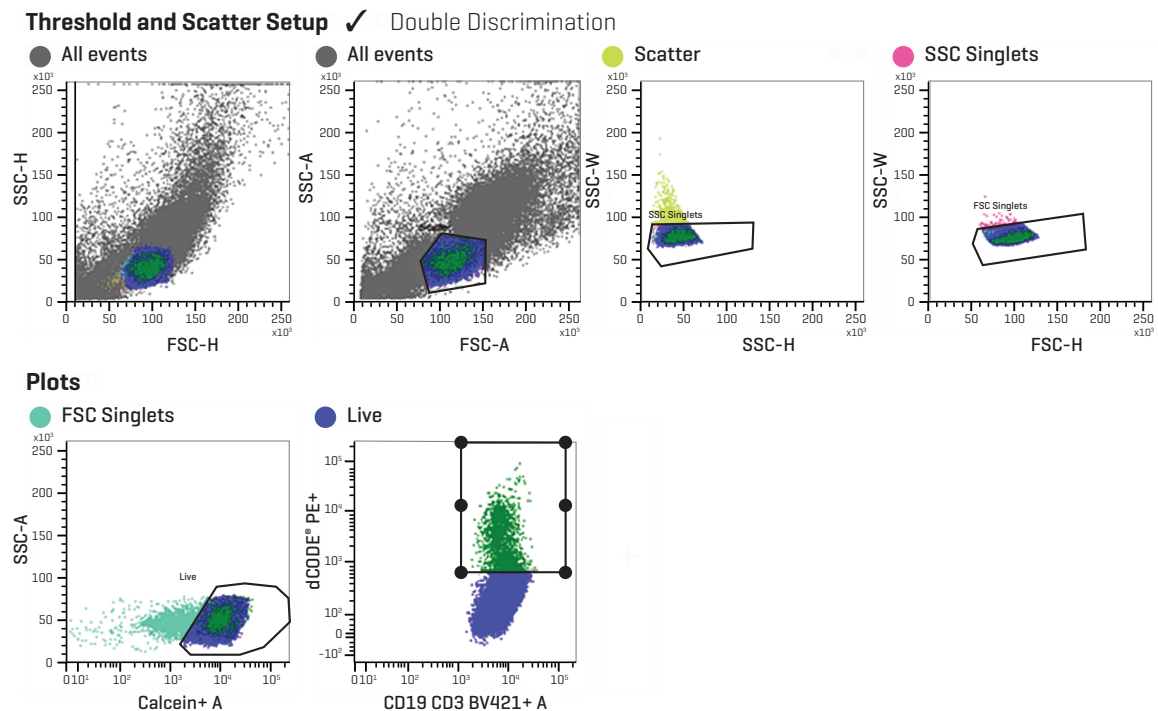


**Sample processing workflow from collection of human PBMCs**



**Fig. 3:** Sample processing workflow from collection of human PBMCs [A] through sorting [B], singularization [C], and generation of libraries for sequencing [D].

## FACS isolation of Ligand-Specific MAIT Cells



**Fig. 4:** Gating strategy for fluorescence-activated sorting (FACS) of cells selected for singularization [input cells]. Cells were gated using sideways (SSC) and forward scatter (FSC). A subsequent gate sorted live cells, among which 5-OP-RU MR1 dCODE Dextramer®-positive and either CD3<sup>+</sup> or CD19<sup>+</sup> were sorted out for loading onto the BD Rhapsody™ cartridge.

## Singularization and library preparation

The BD Rhapsody™ Cartridge Kit was loaded with up to 20,000 input cells, as recommended by the manufacturer [Fig. 3C]. As this was a larger study examining multiple immune cells, various lymphocyte types were loaded into the cartridge, including MAIT cells. Optimal bead-loading efficiency, cell viability, and cell multiplet rates were controlled using the BD Rhapsody™ Scanner. Capture efficiency in the cartridge was 100%. The cells were then lysed to bind mRNA and the oligonucleotides of the MR1 dCODE Dextramer® reagents and AbSeq antibodies onto magnetic beads. The cDNA generated from these sequences was pooled into a LoBind® tube and four libraries were prepared according to the [dCODE Dextramer® \[RiQ\] Library Preparation Protocol: For use with BD Rhapsody™ Single Cell Analysis System and BD protocols for TCR/BCR Full Length Library Preparation](#).

### Optimal loading of the BD Rhapsody™ Cartridge kit

The BD Rhapsody™ Cartridge kit can be loaded with as few as 500 and as many as 20,000 cells. Loading more cells leads to a Poisson distribution of cell multiplets in the wells.

## Sequencing and data analysis

The libraries were sequenced by Novogene on an Illumina® NovaSeq 6000 system and the resulting sequencing data reads were checked on the Seven Bridges Genomics platform. Further analyses and visualizations of gene expression, surface marker expression, and TCR sequences were completed with the BD® SeqGeq™ software [version 2.0].

### MR1 dCODE Dextramer® reference file

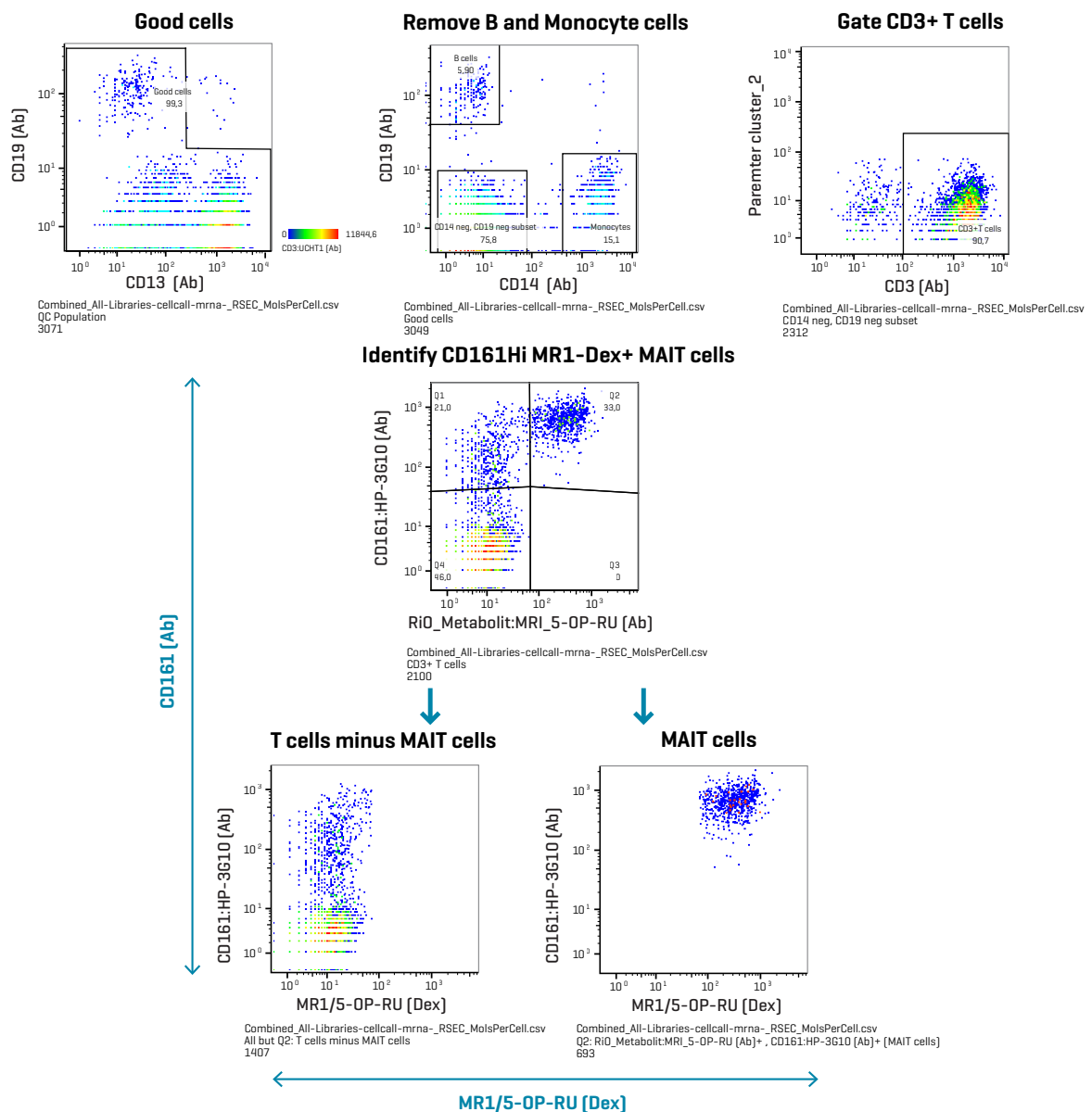
MR1 dCODE Dextramer® reagents come with a reference file containing their DNA barcode ID for easy identification in sequencing data.

## Results and Discussion

### Ligand-specific MAIT cells are accurately identified within the sequencing data

Leveraging the barcodes of the BD<sup>®</sup> AbSeq antibodies, we separated a well-defined population of MAIT cells as CD161<sup>high</sup> 5-OP-RU MR1 dCODE Dextramer<sup>®</sup>-positive cells among CD3<sup>+</sup> T cells. Fig. 5 shows that separation scheme. First, CD3<sup>+</sup>CD19<sup>+</sup> [double-positive] cells were removed to select 3049 cells identified as CD3<sup>+</sup> or CD19<sup>+</sup> and 5-OP-RU MR1 dCODE Dextramer<sup>®</sup>-positive [named “good cells”]. Among those, 180 were B cells (5.9%), 461 were monocytes (15.1%), and 2100 were CD3<sup>+</sup> T cells (68.9%). The remaining 10.1% (308) were other cell types. B cells and monocytes were removed by selecting CD19<sup>-</sup>CD14<sup>-</sup> cells. Then, CD3<sup>+</sup> T cells were selected among the remaining cells. The CD161<sup>high</sup> 5-OP-RU MR1 dCODE Dextramer<sup>®</sup>-positive cells among those CD3<sup>+</sup> T cells were identified as 714 ligand-specific MAIT cells, accounting for 23.4% of all “good cells”. This separation scheme mirrors the surface marker-based gating commonly used to identify MAIT cells by flow cytometry<sup>4</sup> and generated comparable results [Fig. 5]. The agreement underscores the specific performance of the 5-OP-RU MR1 dCODE Dextramer<sup>®</sup> reagent in identifying MAIT cells.

### Identification of ligand-specific MAIT cells in single-cell sequencing data

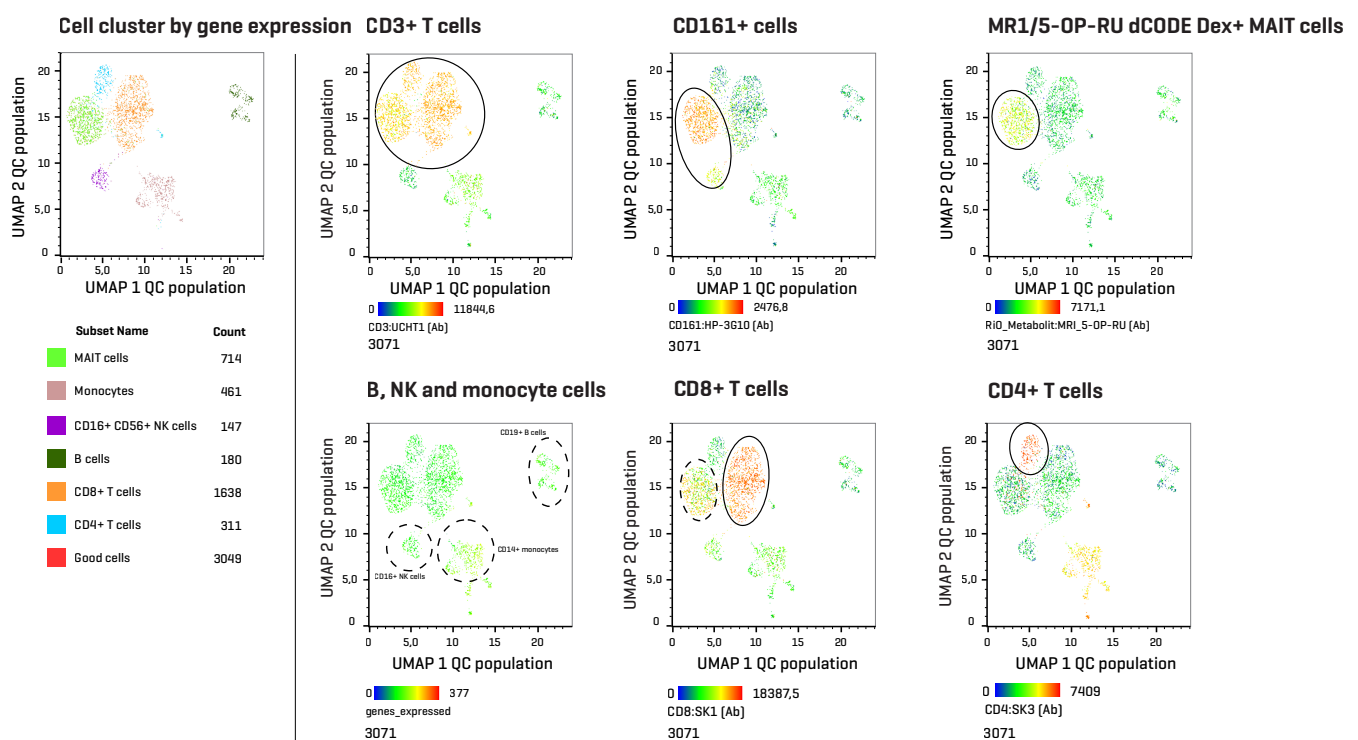


**Fig. 5:** MAIT cells were identified in the single-cell sequencing data by applying a separation scheme akin to surface marker gating in flow cytometry. As a first step, “good cells” were selected by removing the CD3<sup>+</sup>CD19<sup>+</sup> double-positive cells. B cells and monocytes were then excluded from all “good cells” by selecting CD19<sup>-</sup>CD14<sup>-</sup> cells. CD3<sup>+</sup> T cells were then selected among the remaining cells. The MAIT cells were a well-defined population of CD161<sup>high</sup>MR1 dCODE Dextramer<sup>®</sup>-positive cells among the CD3<sup>+</sup> T cells.

## Identify CD161<sup>hi</sup> MR1-Dex<sup>+</sup> MAIT cells

The same well-defined population also emerged in cell clustering based on gene expression analysis. Overlaying the barcode of BD<sup>®</sup> AbSeq antibodies onto cells clustered according to gene expression profiles revealed cell lineages and subpopulations. As expected, the cluster of MAIT cells, recognized as 5-OP-RU MR1 dCODE Dextramer<sup>®</sup>-positive, overlapped with cells that were CD3<sup>+</sup> and CD161<sup>+</sup>. Distinctive about adding 5-OP-RU MR1 dCODE Dextramer<sup>®</sup> to the identification method compared to using only surface markers is that the combination unambiguously discriminates MAIT cells from other CD161-expressing cells, like NK and some T cells [Fig. 6]. Most MAIT cells were also CD8<sup>+</sup>. Staining of the donor PBMCs before cell sorting and single-cell RNA sequencing was clearly successful and the 5-OP-RU MR1 dCODE Dextramer<sup>®</sup> reagent worked well with the BD Rhapsody<sup>™</sup> System.

## Cell clustering based on gene expression profiles



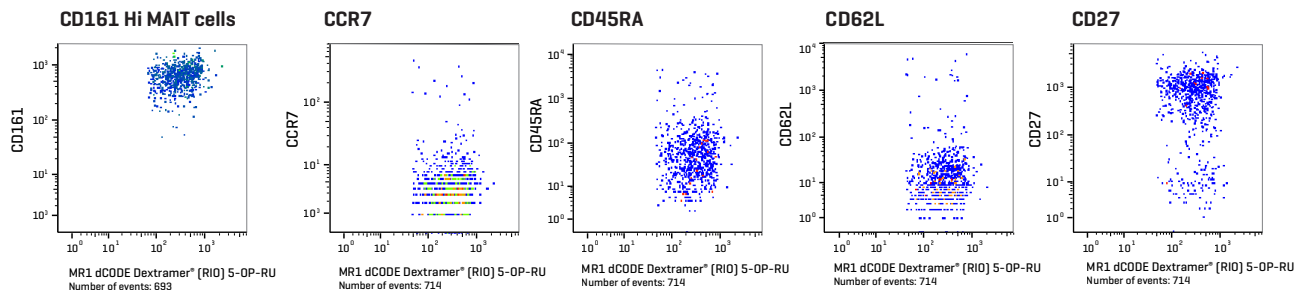
**Fig. 6:** Cell clusters based on gene expression profiles correspond with lineage and subpopulation-specific cell surface markers. MAIT cells are a clearly defined population of CD3<sup>+</sup> CD161<sup>+</sup> 5-OP-RU MR1 dCODE Dextramer<sup>®</sup>-positive cells [top row], most of which also stain for CD8<sup>+</sup> [bottom row, middle].

## Blood MAIT cells have a memory phenotype

Previous studies have shown tissue-resident CD103<sup>+</sup>CD69<sup>+</sup> MAIT cells to exhibit an “alerted” activation state.<sup>4</sup> They express higher levels of the activation markers CD25, HLA-DR and CD38 compared to non-resident MAIT cells. By contrast, MAIT cells in human adult blood express surface markers suggestive of an effector memory phenotype, specifically CD45RO<sup>+</sup>CD27<sup>+</sup>CCR7<sup>-</sup>CD44<sup>high</sup>CD62L<sup>low</sup>.<sup>2-4</sup> The MAIT cells characterized in this study expressed the surface marker profile CCR7<sup>-</sup>CD45RA<sup>low</sup>CD62L<sup>low</sup> [Fig. 7], pointing at the same memory phenotype and readiness for expansion and survival [CD27<sup>+</sup>]. Furthermore, they were CD137<sup>-</sup>CD25<sup>-</sup>HLA-DR<sup>-</sup> with a medium expression of PD-1, indicating a non-activated but non-exhausted state, fit to mount an effective immune response.



## MAIT cell surface marker analysis



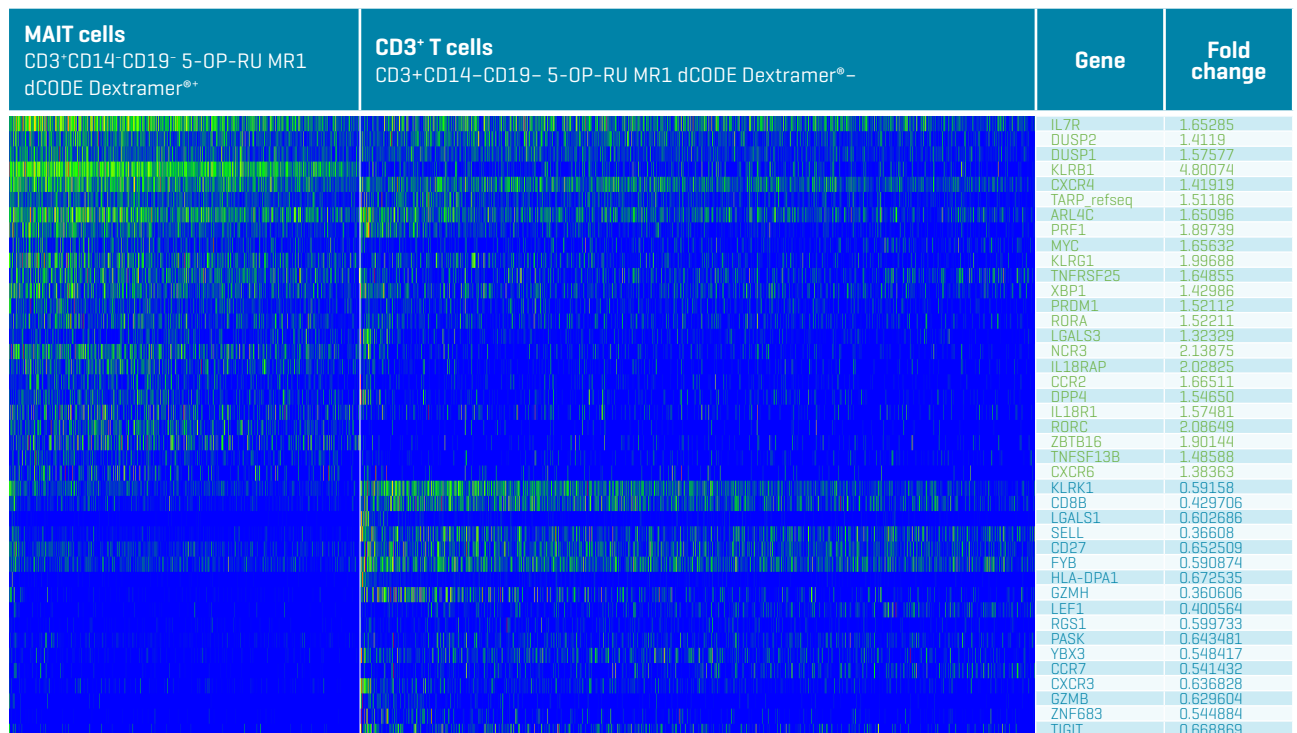
**Fig. 7:** MAIT cells examined for the expression of surface markers show a memory phenotype. The observed profile CCR7<sup>+</sup>CD45RA<sup>low</sup>CD62L<sup>low</sup>CD27<sup>+</sup> corroborates the effector memory phenotype observed for circulating MAIT cells in previous studies.

## Gene expression differs between MAIT cells and other CD3<sup>+</sup> T cells

We compared gene expression levels between MAIT cells and CD3<sup>+</sup> T cells. Specifically, these were CD3<sup>+</sup>CD14<sup>-</sup>CD19<sup>-</sup> 5-OP-RU MR1 dCODE Dextramer<sup>+</sup>-positive cells [MAIT cells] compared to all CD3<sup>+</sup>CD14<sup>-</sup>CD19<sup>-</sup> 5-OP-RU MR1 dCODE Dextramer<sup>-</sup>-negative cells remaining from the last gate in the separation scheme illustrated in Fig. 4. Fig. 8 shows the top up- and downregulated genes identified in the MAIT cell population compared to the remaining CD3<sup>+</sup> T cells. Several of the differentially expressed genes were immune-related, indicative of the unique response of MAIT cells to different immunological environments and signals.

Garner *et al.* compared gene expression in blood MAIT cells versus memory T cells defined as CD3<sup>+</sup>CCR7<sup>+</sup>MR1/5-OP-RU<sup>-</sup>.<sup>7</sup> Thirty-two of the 40 genes highlighted in this study were also identified in the Garner study, with changes in all but one of the genes in the same direction [up- or downregulation]. That gene was CD27. We did not discriminate among T cell phenotypes in the comparison group. Thus, it likely included naïve, regulatory, and central memory T cells.<sup>10</sup> Garner *et al.* excluded CCR7<sup>+</sup> T cells from their analysis. This restriction to a memory phenotype may account for the observed difference.

## MAIT cell and CD3<sup>+</sup> T cell differential gene expression



**Fig. 8:** MAIT cells show differential gene expression compared to CD3<sup>+</sup> T cells. Both upregulated [top, green names] and downregulated sets [bottom, blue names] include genes involved in immune functions.

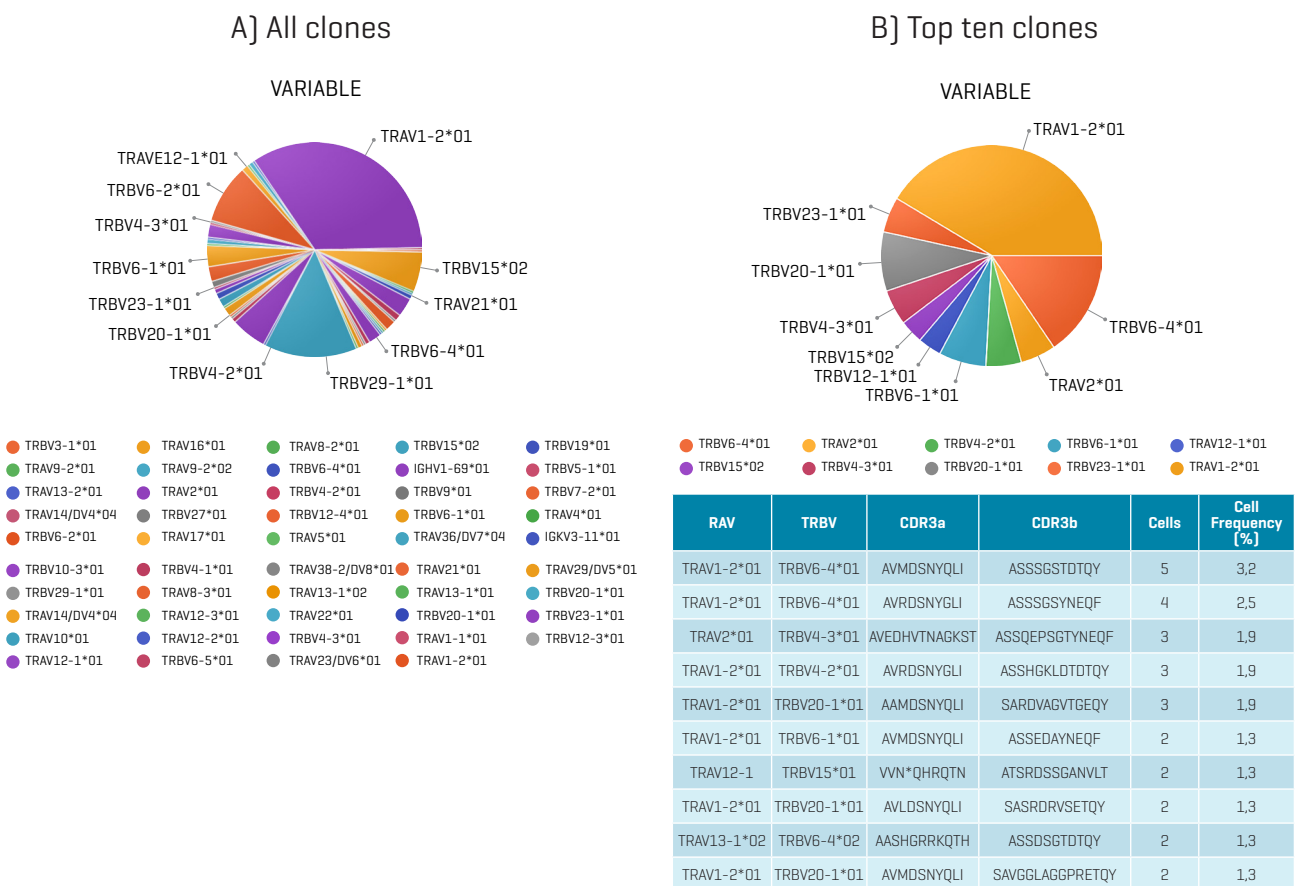
low high

## TCR sequence in MAIT cells shows high variation

V(D)J analysis revealed that the 5-OP-RU-specific MAIT cells had restricted V and J gene segment usage (Fig. 9). In the  $\alpha$  chain, TRAV1-2 and TRAJ33 (not shown in Fig. 9) were most common across the whole MAIT cell population and dominated the most frequent 10 clonotypes. Restriction was less apparent in the  $\beta$  chain. Although TRBV6 and TRBV20 were the most common V gene segments among all clonotypes, several other sequences were also represented. These results are aligned with previously published data.<sup>3,4,7</sup>

Despite the restricted gene segment usage, overall paired TCR sequence variation was high, with 138 clonotypes in 158 cells. That variability stemmed primarily from differences in the  $\beta$  chain, both in the repertoire of V gene segments used and, in the sequence, and length of the CDR3 region (Fig. 9B). Furthermore, the MAIT cell population exhibited no major clonotypic dominance. Garner *et al.* made similar observations, concluding that the TCR $\beta$  chain “governs the uniqueness of individual MAIT cell TCR repertoires”.<sup>7</sup>

## MAIT cell TCR clonotype profiling



**Fig. 9:** TRAV and TRBV gene segment representation in all TCR clonotypes (A) and the top 10 most frequent clonotypes (B) of the analyzed MAIT cell population. Although both  $\alpha$  and  $\beta$  chains of the TCR showed restricted gene segment usage, overall variability in TCR clonotypes was high, with even the 10 most common clonotypes found in a small fraction of the cell population (B).

As research on MAIT cells increases, the picture emerging is that of a population of unconventional T cells with the ability to sense a range of immunogenic cues and to respond in equally broad immune roles, be it direct defense against pathogens, triggering inflammation, supporting cellular immunity, or aiding wound healing.<sup>11</sup> While some studies connect this functional flexibility to transcriptional profiles of MAIT cells associated with “killer,” “helper,” and “regulatory” phenotypes,<sup>8</sup> other work points at a single population of cells with simply a broad spectrum of response options.<sup>7</sup> Either way, TCR clonotype is a factor in the distinctive behavior of a MAIT cell. Clonal identity influences the cell’s resting and activated transcriptional profile and activation potential.<sup>7</sup> The sensitive detection and analysis of 5-OP-RU-specific MAIT cells achieved in this study showed that their paired TCR molecules were highly variable and each clonotype accounted for just a small fraction of the overall cell population. Understanding that variability and its influence on MAIT cell activity will prove instrumental in using these cells as therapeutic agents.

## Conclusion

- This study demonstrated that MR1 Dextramer® products achieve sensitive and specific detection of MAIT cells and, when used with the BD Rhapsody™ System, facilitate single-cell multi-dimensional analyses that reveal correlations between phenotype, TCR sequence, and gene expression.
- The 5-OP-RU MR1 dCODE Dextramer® reagent reliably and specifically identified CD3<sup>+</sup>CD8<sup>+</sup>CD161<sup>high</sup> MAIT cells on the BD Rhapsody™ System. Data from relevant cell surface markers and 5-OP-RU MR1 dCODE Dextramer® reagent matched cell clustering by gene expression and allowed the separation of a well-defined population of 5-OP-RU-specific CD3<sup>+</sup>CD8<sup>+</sup>CD161<sup>high</sup> MAIT cells.
- Cell surface marker expression data corroborated evidence from the literature that 5-OP-RU-specific blood MAIT cells exhibit a memory phenotype [CCR7<sup>-</sup>CD45RA<sup>low</sup>CD62L<sup>low</sup>] and a non-active [CD137<sup>-</sup>CD25<sup>-</sup>HLA-DR<sup>-</sup>] but non-exhausted and response-ready state [CD27<sup>high</sup>PD-1<sup>med</sup>].
- The differential gene expression of 5-OP-RU-specific MAIT cells compared to other CD3<sup>+</sup> T cells reflected their unique response to immunological environments and signals. These data can be examined in connection with dominant or low-abundance TCR clonotypes at single-cell resolution.
- The V[D]J analysis and examination of CDR3 usage confirmed the reported semi-invariant TCR α chain of MAIT cells. TRAV1-2, TRAJ33, TRBV6, and TRBV20 were the most used gene segments, in agreement with the literature. Nevertheless, 137 clonotypes were identified among 158 cells with paired TCRs, and the 10 most frequent clonotypes comprised a minor fraction of the overall population [1.3-3.2%]. The TCR repertoire of the MAIT cells was diverse, with no major clonotypic dominance.

## Appendix

**Supplementary Table 1. BD® AbSeq antibodies to phenotype detected antigen-specific T cell populations**

BD® AbSeq Antibodies			
Specificity	Clone	Oligo ID	BD® Cat. no.
CD3	UCHT1	AHS0231	625970
CD4	SK3	AHS0032	625970
CD8	SK1	AHS0228	625970
CD11c	B-Ly6	AHS0056	625970
CD14	MPHIP9	AHS0037	625970
CD16	3G8	AHS0053	625970
CD19	SJ25C1	AHS0030	625970
CD25	2A3	AHS0026	625970
CD27	M-T271	AHS0025	625970
CD28	L293	AHS0138	625970
CD45RA	HI100	AHS0009	625970
CD56	NCAM16	AHS0019	625970
CD62L	DREG-56	AHS0049	625970
CD127	HIL-7R-M21	AHS0028	625970
CD134	ACT35	AHS0013	625970
CD137	4B4-1	AHS0003	625970
CD161	HP-3G10	AHS0205	625970
CD183 [CXCR3]	1C6/CXCR3	AHS0031	625970
CD185 [CXCR5]	RF8B2	AHS0039	625970
CD186 [CXCR6]	13B 1E5	AHS0148	625970
CD196 [CCR6]	11A9	AHS0034	625970
CD197 [CCR7]	2-LT-A	AHS0273	625970
CD272	J168-540	AHS0052	625970
CD278	DX29	AHS0012	625970
CD279	EH12.1	AHS0014	625970
CD357 [GITR]	V27-580	AHS0104	625970
CD366 [TIM-3]	7D3	AHS0016	625970
HLA-DR	646-6	AHS0035	625970
IgD	IA6-2	AHS0058	625970
IgM	G20.127	AHS0198	625970

## References

1. Porcelli, S. *et al.* [1993] Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4<sup>+</sup>CD8<sup>-</sup> alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. *J. Exp. Med.* DOI: [10.1084/jem.178.1.1](https://doi.org/10.1084/jem.178.1.1)
2. Hicks, T.S.C. and Zhang, X-W. [2020] MAIT cell activation and functions. *Front. Immunol.* DOI: [10.3389/fimmu.2020.01014](https://doi.org/10.3389/fimmu.2020.01014)
3. Godfrey, D.O. *et al.* 2019. The biology and functional importance of MAIT cells. *Nature Immunol.* DOI: [10.1038/s41590-019-0444-8](https://doi.org/10.1038/s41590-019-0444-8)
4. Nel, I. *et al.* [2021] MAIT cells, guardians of skin and mucosa? *Mucosal Immunol.* DOI: [10.1038/s41385-021-00391-w](https://doi.org/10.1038/s41385-021-00391-w)
5. Corbett, A.J. *et al.* Antigen recognition by MR1-reactive T cells; MAIT cells, metabolites, and remaining mysteries. *Front. Immunol.* DOI: [10.3389/fimmu.2020.01961](https://doi.org/10.3389/fimmu.2020.01961)
6. Yao, T. *et al.* [2020]. Leveraging Public Single-Cell and Bulk Transcriptomic Datasets to Delineate MAIT Cell Roles and Phenotypic Characteristics in Human Malignancies. *Front. Immunol.* DOI: [10.3389/fimmu.2020.01691](https://doi.org/10.3389/fimmu.2020.01691)
7. Garner, L.C. *et al.* [2023] Single-cell analysis of human MAIT cell transcriptional, functional and clonal diversity. *Nature Immunol.* DOI: [10.1038/s41590-023-01575-1](https://doi.org/10.1038/s41590-023-01575-1)
8. Vorkas, C.K. *et al.* [2022] Single-cell transcriptional profiling reveals signatures of helper, effector, and regulatory MAIT cells during homeostasis and activation. *J. Immunol.* DOI: [10.1093/jimmunol.2100522](https://doi.org/10.1093/jimmunol.2100522)
9. Keller, A.N. *et al.* [2017] Drugs and drug-like molecules can modulate the function of mucosal-associated invariant T cells. *Nature Immunol.* DOI: [10.1038/ni.3679](https://doi.org/10.1038/ni.3679)
10. Förster, R. *et al.* [2008] CCRT and its ligands: balancing immunity and tolerance. *Nature Rev. Immunol.* DOI: [10.1038/nri2297](https://doi.org/10.1038/nri2297)
11. Provine, N.M. and Klenerman, P. [2020] MAIT cells in health and disease. *Ann. Rev. Immunol.* DOI: [10.1146/annurev-immunol-080719-015428](https://doi.org/10.1146/annurev-immunol-080719-015428)

## Resources

### MR1 Dextramer

- Reliable detection of ligand-specific MAIT cells

Learn more: [immudex.com/MR1-dextramer](https://immudex.com/MR1-dextramer)

### TCR Discovery

Explore how TCR discovery can advance the development of novel T-cell therapies.

Learn more: [immudex.com/tcr-discovery](https://immudex.com/tcr-discovery)

### Case Studies and Application Notes

Immerse yourself in educational content exploring the applications of Dextramer® technology.

Learn more: [immudex.com/education](https://immudex.com/education)

### MR1 dCODE Dextramer®

- Deep characterization of ligand-specific MAIT cells by NGS or single-cell multi-omics

Learn more: [immudex.com/MR1-dCODE](https://immudex.com/MR1-dCODE)

### MR1 Monomers

- Biotinylated soluble MR1 Monomers for your MAIT cell research

Learn more: [immudex.com/MR1-monomers](https://immudex.com/MR1-monomers)

## Contact us

### Immudex ApS

Bredevej 2A  
2830 Virum  
Denmark

Email: [customer@immudex.com](mailto:customer@immudex.com)

Tel.: +45 29 13 42 24

For research use only. Not for use in diagnostic or therapeutic procedures.

© Immudex ApS. Denmark, 2023

BD, BD Rhapsody, AbSeq and SeqGeek are trademarks or registered trademarks of Becton, Dickinson and Company or its affiliates. BV421 is equivalent to Brilliant Violet™ 421, which is a trademark or registered trademark of Becton, Dickinson and Company or its affiliates, and is used under license.

Immudex is the sole manufacturer and provider of dCODE Dextramer® (RiO) reagents, and support related to these products is through Immudex.