

dCODE Dextramer® (10x) Package Insert

Products dCODE Dextramer® (10x), Cat. No. WBxxxxxdXG / FBxxxxxdHG
 CD1d dCODE Dextramer® (10x), Cat. No. XDxxxxxDXG / YDxxxxxDXG
 MR1 dCODE Dextramer® (10x), Cat. No. ZAxxxxxDXG / ZBxxxxxDXG
 HLA-E dCODE Dextramer® (10x), Cat. No. URxxxxxdXC
 HLA-G dCODE Dextramer® (10x), Cat. No. USxxxxxdXG
 Collectively denominated dCODE® (10x) reagents.

Recommended use Single-cell multi-omics analysis of antigen-specific T, NKT, MAIT, or NK cells in cell samples, using the 10x Chromium Single Cell Gene Expression Platform.

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

Description dCODE® (10x) reagents consist of a dextran polymer backbone carrying multiple MHC, CD1d, MR1, HLA-E, or HLA-G-antigen complexes, and R-phycoerythrin (PE) for sorting of dCODE® (10x) positive cells before loading the sample into the 10x Chromium platform. In addition, each dCODE Dextramer® has a DNA oligonucleotide attached with a DNA barcode sequence that defines the monomeric MHC-antigen complexes on the Dextramer®.

- dCODE® (10x) reagents are provided at a concentration of 160 nM in PBS, containing 1% bovine serum albumin (BSA) and 15 mM NaN₃, pH 7.2.
- 2 µL (1 test) is recommended for staining of 1-3 x 10⁶ PBMCs.
- Each dCODE® (10x) reagent is uniquely identified by its allele / Peptide / DNA Barcode.

The Unique DNA Barcode comprises:

- Primer sequence compatible with Illumina® Sequencers (Nextera pR2)
- Unique molecule identifier (UMI)
- ID sequence (barcode) that specify the MHC-peptide or MR1-ligand specificity
- Capture sequence for 10x Chromium single cell immune profiling solution.

5' -Nextera pR2 - - UMI - - ID - - UMI - - Capture seq - 3'
 CGGAGATGTGTATAAGAGACAGNNNNNNNNNNXXXXXXXXXXXXXXXXNNNNNNNNNNCCCATATAAGAAA 3'

Critical Our DNA barcodes are not unique to a given dCODE Dextramer® specificity but is assigned randomly during production. It is crucial, during planning of an experiment involving multiple dCODE Dextramer® products, to ascertain that their barcode sequences are different.

Sizes dCODE® (10x) reagents - Gold: Single reagents of 25 tests (50 µL), 50 tests (100 µL), or 150 tests (300 µL) each.
 dCODE® (10x) reagents - Explore: Panels of 16, 32, 48, 64, 80, or 96 dCODE® (10x) reagents for 10 tests (20 µL), 25 tests (50 µL), or 50 tests (100 µL) each.

Storage	<p>dCODE® (10x) reagents should be stored at 2-8°C in the dark – the plastic vial only partially protects the reagents against light.</p> <p>HLA-E dCODE Dextramer® should be kept at 2-8°C or on ice during general handling of the reagent.</p>
Precautions	<p>Contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.</p>
Patents	<p>The dCODE® technology is disclosed in granted and pending patents within the WO 2015/185067 and WO 2015/188839 patent families including US11402373, US11585806, US11668705, EP3152232, EP3155426, HK1236546 B, AU2015271324, AU2019264685, AU2021204496, CA2951325, SG11201610177U, JP6956632 and JP7271465.</p>
Symbols	<p>See www.immudex.com/symbols</p>
Manufacturer	<p>Immudex, Bredevej 2A, DK-2830 Virum, Denmark</p>
Materials Provided	<p>MHC dCODE Dextramer® (10x) and/or CD1d dCODE Dextramer® (10x), MR1 dCODE Dextramer® (10x), HLA-E dCODE Dextramer® (10x), HLA-G dCODE Dextramer® (10x)</p>
Materials Required (not provided)	<p>Stain buffer: PBS, pH 7.4 containing 1-5% Serum and 0.1 g/L Herring sperm DNA</p> <p>Wash buffer: PBS, pH 7.4 containing 1-5% Serum</p> <p>Antibodies identifying relevant cell surface markers: For CD8⁺ T, CD4⁺ T and NKT cells (e.g., CD3, CD4 and CD8). For MAIT cells (e.g., CD3, CD4, CD8 and CD161). Optionally other desired antibodies and live-dead dye.</p>
Procedure	<p>Staining with dCODE® (10x) must be kept shielded from light.</p> <ol style="list-style-type: none"> 1. Prepare PBMC sample and resuspend cells in stain buffer. 2 µL dCODE® (10x) reagent is enough to stain 1-3 x 10⁶ PBMC in a volume of 50 – 100 µL. 2. Preparation of dCODE® (10x) reagent pool: <ol style="list-style-type: none"> a. In an empty tube, add 0.2 µL 100µM d-Biotin per dCODE® (10x) specificity b. Add 2 µL of each dCODE® (10x) specificity, mix 3. Add the pool of dCODE® (10x) reagents to the cell sample and mix thoroughly 4. Incubate in the dark at room temperature: <ol style="list-style-type: none"> a. MHC I, CD1d, MR1, HLA-E^A, and HLA-G dCODE Dextramer® (10x) pool: 10 min. incubation^B b. MHC II dCODE Dextramer® (10x) pool: 30 min. incubation^B c. dCODE® (10x) pool comprised of a. and b: 30 min. incubation^B. 5. Add relevant antibodies in volume and concentration recommended by provider. Incubate for 20 min.

NB: If no antibodies are added continue incubation with dCODE Dextramer® reagent pool for additional 20 min.

6. Washing:
 - a. If staining in 4 mL tubes, add 2 mL wash buffer. Centrifuge at 300 x g for 5 min. and remove the supernatant. Repeat washing with additional 2 mL wash buffer
 - b. If staining in 96-well microtiter plates, make 4 sequential washes using 200 µL wash buffer per well. Centrifuge at 300 x g for 5 min. between each wash and remove supernatant.
7. Resuspend cells in adequate volume (as recommended by 10x Genomics) of either of the buffers:
 - a. PBS (Calcium, Magnesium free) + 0.04 % W/V BSA, or
 - b. PBS, Dulbecco's Phosphate-Buffered Saline (DPBS), Hank's Balanced Salt Solution (HBSS), Eagle's Minimum Essential Medium (EMEM), or Dulbecco's Modified Eagle Medium (DMEM) + 10% FBS.
8. Proceed to the 10x Genomics Feature Barcode Protocol:
 - [CG000208 Chromium Next GEM Single Cell V\(D\)J Reagent Kit Feature Barcoding technology](#)
 - [CG000126 Cell counting flowchart](#)
 - [Single Cell Protocols - Cell Preparation Guide](#)

Procedural Notes

- A. HLA-E dCODE Dextramer® should be kept at 2-8°C or on ice during general handling of the reagent, while staining is performed at room temperature.
- B. Incubation time may be increased when using a high number of reagents in pool staining and requires optimization.

Notes

Cell viability is crucial for successful Single Cell Immune Profiling assay. We recommend determining the cell concentration and viability, of your cell sample after staining and before introducing the cells into the Chromium chip. Cell viability should be >70%.

Antigen-specific T cells are of low frequency in peripheral blood. Enough cells must be stained and analysed for detecting rare cell populations. Alternative, a pre-enrichment step may be introduced to enrich the dCODE® (10x) positive cells, before applied to the Chromium Chip.

Read more about Single Cell Immune Profiling, and find relevant support tools at

- www.10xgenomics.com
- [Single Cell Immune Profiling Support](#)
- [Immune Profiling Feature Barcoding](#)

Technical Support

For additional Tips & Tricks, FAQs and protocols, please visit <https://www.immudex.com/resources/>

Immudex® is the sole manufacturer and provider of dCODE Dextramer® (10x) reagents, and support related to these products is through Immudex.

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