# dCODE Dextramer® (RiO)

**dCODEDextramer® (RiO) - Gold** **Cat. No. dCR**
**dCODEDextramer® (RiO) - Explore** **Cat. No. dCRE**

**Recommended use**

Profiling and quantitation of antigen-specific T cells in cell samples, using the BD Rhapsody™ Single-Cell Analysis System.
For research use only. Not for use in diagnostic or therapeutic procedures. Immudex is the sole manufacturer and provider of dCODE Dextramer® (RiO) reagents, and support related to these products is through Immudex.

**Reagents provided**

dCODE Dextramer® (RiO) reagent consists of a dextran polymer backbone, carrying multiple MHC-peptide complexes, a corresponding unique DNA Barcode oligo and R-phycoerythrin (PE) for sorting of dCODE Dextramer® positive cells before loading the sample into the BD Rhapsody™ system.

The unique DNA Barcode oligo comprises:
BD Rhapsody™ compatible PCR handle sequences for PCR amplification
Unique Molecule Identifier (UMI) sequence
DNA Barcode sequence that specifies the MHC-peptide specificity


dCODE Dextramer® (RiO) is provided at a concentration of 1.6 x $10^{-7}$ M in PBS buffer, containing 1% bovine serum albumin (BSA) and 15 mM NaN3, pH 7.2.

2 µl (one test) is recommended for staining of 1-10 x $10^6$ PBMC.

Each dCODE Dextramer® is uniquely identified by its HLA-allele / Peptide / DNA Barcode.

**Required reagents not provided**

Reagents for use with the BD Rhapsody™ Single-Cell Analysis System must be ordered from BD Biosciences. Please see protocol below for detailed description.

For preperation of the dCODE® DNA library, dCODE® specific PCR primers are required:
dCODE PCR1 primer: 5'-GGAGGGAGGTTAGCGAAGGT-3'
dCODE PCR2 primer: 5'-CAGACGTGTGCTCTTCCGATCTGGAGGGAGGTTAGCGAAGGT-3'

dCODE® specific primers can be ordered from a preferred DNA oligo provider and are used at a concentration of 10 µM.

**Sizes**

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

**Storage**

Store in the dark at 2-8°C.

**Precautions**

Contains sodium azide ($NaN_3$), 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.
As with any product derived from biological sources, proper handling procedures should be used.
For professional users.

**References**

Multi-omics characterization of T-cell populations at the single-cell level utilizing sensitive dCODE Dextramer® and BD® AbSeq Assay on the BD Rhapsody™ Single-Cell Analysis System (Poster presented at SITC 2020).

**Patents**

The dCODE Dextramer® and its use are disclosed in granted and pending patents within the WO 2015/185067, WO 2015/188839 and WO 2002/072631 patent families.

**Symbols**

See www.immudex.com/symbols

**Technical support**

E-mail Immudex customer support: customer@immudex.com

Phone Immudex Denmark: +45 3110 9292

**Manufacturer**

Immudex, Bredevej 2A, DK-2830 Virum, Denmark

## dCODE Dextramer® (RiO) staining protocol

**Materials required (not provided)**

Cell labeling buffer: PBS, pH 7.4 containing 1-5% serum and 0.1 g/l herring sperm DNA (shirred) or BD™ Stain Buffer (FBS) (Cat. No. 554656), added 0.1 g/l herring sperm DNA (shirred)
Wash buffer: PBS, pH 7.4 containing 1-5% serum
100 µM d-Biotin in PBS
Oligo conjugated, BD® AbSeq Assay (optional)
Fluorescent markers for FACS sorting: (As needed)

**Cell staining procedure**

Prepare single cell PBMC sample

1. Resuspend 1-3 x $10^6$ PBMC in 50 µl cell labeling buffer.
    a. If a viability stain is used in the sorting, resuspend cells in 1 ml wash buffer (azide free) and add recommended volume of viability stain.
    Incubate for 15 min at RT (all incubations must be performed shielded from light).
    Add 2 ml Wash buffer, centrifuge 300-600 x g for 5 min, resuspend cells in 50 µl cell labeling buffer and continue to step 2.

Prepare dCODE Dextramer® pool and label cells with dCODE Dextramer®
*(Cell labeling with dCODE Dextramer® must always be performed before staining with antibodies)*

2. Centrifuge dCODE Dextramer® (RiO) at 10,000 x g for 1 min.
3. Add 0.2 µl 100 µM d-Biotin per dCODE Dextramer® specificity into an empty Falcon® tube, 5 ml Round Bottom Polystyrene Test Tube (Corning Cat. No. 352054).
4. Add 2 µl of each dCODE Dextramer® specificity and mix.
*Note: Pooled dCODE Dextramer® reagents must be used within 1-2 hour after mixing.*
5. Add the pool of dCODE Dextramer® reagents to the cell sample and mix.
6. Incubate at room temperature for 10 min[3] shielded from light.
- While incubating, prepare 2x BD® AbSeq antibody master mix (optional).

Prepare 2X BD® AbSeq labeling mix[2]

7. Centrifuge BD® AbSeq antibody tubes at 400 × g for 30 sec and place on ice.
8. In pre-amplification workspace, pipett the BD® AbSeq antibodies into a new 1.5 ml LoBind Eppendorf Tube on ice:

| Component | (N = no. antibodies) 1 sample | 1<N<40 add 30% overhead |
|---|---|---|
| Per BD® AbSeq Ab-Oligo | 2 µl | 2,6 × N µl |
| BD Stain Buffer (FBS) (Cat. No. 554656) | 100 µl – (2×N) | 100 µl – (2,6 × N) |
| Total | 100 µl | 130 µl |

9. Pipet-mix the 2X BD® AbSeq labeling master mix and place on ice.

BD® AbSeq labeling

10. Bring the volume of the labeling reaction up to 100 µl adding cell labeling buffer (if labeling reaction is >100 µl skip this step).
11. Add the BD® AbSeq 2x pool to the sample and mix by pipetting.
12. Add the sorting antibody conjugates. Use a volume of antibody as recommended by the manufacture and mix by pipetting.
13. Incubate at 4°C for 30-60 min shielded from light.

Wash labeled cells and sort

14. If staining is performed in 4 ml Falcon® tubes, add 2 ml Wash buffer.
15. Centrifuge at 300-600 x g for 5 min and remove the supernatant. For highest cell retention, invert to decant supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.
Repeat for a total of 3 times.
    a. If labeling in 96-well microtiter plates, make 6 sequential washes using 200 µl wash buffer per well. Centrifuge at 300-600 x g for 5 min between each wash and remove supernatant.
16. Resuspend cells in adequate volume of Wash buffer and store sample on ice until sorting is performed.
- If not performing cell sorting, go directly to step 20.
17. Dextramer® positive cells are sorted by flow cytometry, using the PE-fluorescence of the dCODE Dextramer® following the guidelines and practices of your sorting facility.
*Use sort mode for "Yield". If "purity" mode is used less cells will be sorted.*
18. Collect sorted cells directly into a tube containing suitable buffer.
*Viability is increased if the final sorted cell is in a buffer containing >10% serum.*
Keep the unsorted and sorted cells at 4°C while performing the cell sorting.

19. Centrifuge the sorted cell sample 300-600 x g for 5-10 min (depending on the sorting volume), invert to decant supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.

Go immediately to step 20, do not pause the procedure here!

**Single cell capture and cDNA preparation using the BD Rhapsody™ Express Single-Cell Analysis System**

20. Perform single cell capture and cDNA library preperation, following the BD protocol:
" Single Cell Capture and cDNA Synthesis with the BD Rhapsody™ Single-Cell Analysis System" Doc ID: 210966".

**DNA library preparation**

This part provides instructions on creating the dCODE® DNA library in combination with targeted mRNA and optional BD® AbSeq DNA library.

The dCODE® library preparation protocol[a] is an addendum to BD's "mRNA Targeted and BD® AbSeq Library Preparation with the BD Rhapsody™ Targeted mRNA and BD® AbSeq Amplification Kit" (Doc ID: 214293)[b].

BD® AbSeq Assay library preparation can be obmitted from the protocol.

Both protocols should be read carefully before proceeding to step 21.

21. Proceed with DNA library preparation, using the two protocols:
    a. dCODE Dextramer® (RiO) library preparation protocol (TF1196.01).
    b. "mRNA Targeted and BD® AbSeq Library Preparation with the BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit" (Doc ID: 214293).

**Staining protocol notes**

1. Cell labeling with dCODE Dextramer® must always be performed before staining with antibodies.
2. BD™ Biosciences' recommendations:
   - Creating freshly pooled antibodies before each experiment.
   - Creating reagent pools with 20% overage to ensure adequate volumes for labeling.
   - This protocol is based on using HPBMC (human peripheral blood mononuclear cells).
3. Incubation time should be increased for larger pools of dCODE Dextramer® reagents. For more than 25 specificities, use 20-30 min incubation.

**Sequencing requirements**

For sequencing of the dCODE® library, follow the requirement and recommendations as for BD® AbSeq in the "mRNA Targeted and BD™ AbSeq Library Preparation with the BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit, protocol" ( Doc ID: 214293).

**BD Rhapsody™ protocols**

Doc ID: 210966: Single Cell Capture and cDNA Synthesis with the BD Rhapsody™ Single-Cell Analysis System.

Doc ID: 214293: BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit.

Note: Doc ID refers to BD protocol documentation