Recommended use
Profiling and quantitation of antigen-specific T cells in cell samples\textsuperscript{1,2,3}.
For research use only. Not for use in diagnostic or therapeutic procedures.

Reagents provided
dCODE Dextramer (HiT) reagent consists of a dextran polymer backbone carrying multiple MHC-peptide complexes, a corresponding unique DNA Barcode oligo and R-phycocerythrin (PE) for sorting of dCODE Dextramer positive cells.
The unique DNA Barcode oligo comprises:
- Forward and reverse primer handle sequences for amplification of DNA Barcode
- Unique Molecule Identifier (UMI) sequence
- DNA Barcode sequence that specifies the MHC-peptide specificity

Reverse handle DNA Barcode (18) UMI (18) Forward handle
5' -CTGTGACTATGTAGGCTTTCCNNNNNNNNNNNNNNACGCTGGCTGAAACTTC-3'
dCODE Dextramer (HiT) is provided at a concentration of \(1.6 \times 10^{-7}\) M in PBS buffer containing 1% bovine serum albumin (BSA) and 15 mM NaN\(_3\), pH 7.2.
2 \(\mu\)l/test is recommended for staining of 1-3 \(\times 10^{6}\) PBMC.

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

Sizes
dCODE Dextramer (HiT) - Gold: Single reagents of 25 tests (50 \(\mu\)l), 50 tests (100 \(\mu\)l) or 150 tests (300 \(\mu\)l) each.
dCODE Dextramer (HiT) - Explore: Panels of 16, 32, 48, 64, 80, or 96 dCODE Dextramer (HiT) reagents for 10 tests (20 \(\mu\)l), 25 tests (50 \(\mu\)l) or 50 tests (100 \(\mu\)l) each.

Storage
Store in the dark at 2-8\(^\circ\)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

Patents
The dCODE Dextramer (HiT) and its uses 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 for explanation of symbols.

Technical support
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Telephone: +45 3110 9292 (Denmark)

Manufacturer
Immudex, Bredevej 2A, DK-2830 Virum, Denmark
**dCODE Dextramer® (HiT)**

**Staining Protocol**

**Materials required (not provided)**
- Stain buffer: PBS, pH 7.4 containing 1-5% serum and 0.1 g/l Herring sperm DNA
- Wash buffer: PBS, pH 7.4 containing 1-5% serum
- Sorting buffer: PBS, pH 7.4 containing 50% serum
- 100 µM d-Biotin in PBS, pH 7.4
- Antibodies identifying relevant cell surface markers (e.g. CD3, CD4, CD8)
- Forward and reverse amplification primers. For design of amplification primers, see Appendix A
- qPCR reaction components
- QiAquick® PCR Purification Kit

**Procedure**

1. Prepare PBMC sample and resuspend 1-3 x 10⁶ PBMC in 50-100 µl stain buffer.
2. Centrifuge dCODE Dextramer (HiT) at 10,000 x g for 1 min.
3. Preparation of dCODE Dextramer (HiT) reagent pool:
   a. Add 0.2 µl 100 µM d-Biotin per dCODE Dextramer specificity into an empty tube
   b. Add 2 µl of each dCODE™ Dextramer specificity and mix
   c. From this mixture, take 1 µl and dilute with 99 µl wash buffer. Save this for qPCR amplification
5. Incubate at room temperature for 20 min.
6. Add relevant antibodies in the volume/concentration recommended by provider. Incubate for 20 min.

**Analysis of data**

Demultiplex the sequencing data according to sample (primer barcodes) and Dextramer reagents (oligo barcodes). Calculate the apparent enrichment (AE) for each reagent by dividing its read count in the Dextramer-PE-positive sample with the corresponding read count in the Dextramer-PE-negative sample. To get the specific enrichment, divide each AE with the (median of the) AE of the control Dextramer reagents.

Sequencing reads from the Dextramer input mixture serve to validate the presence of all Dextramer reagents.

**Procedural notes**

Always keep dCODE Dextramer stored at 2-8°C in the dark – the plastic vial only partially protects the reagents against light.
**Appendix A**

**DNA Oligo amplification and Primer design**

![Diagram of PCR amplification and sample barcoding]

**Sequencing**

Note: If the amplified product is sequenced using the A-key, the sequence read corresponds to the reverse complement of the oligo.

Amplification primers:
- Amplification primers are used in the PCR reaction to amplify the target region of the DNA barcode oligo.
- Each primer consists of a region for annealing to the oligo (forward or reverse handle), a unique sample ID sequence to individual label samples for multiplex sequencing, and a sequencing handle (A-key and P-key).
- The A-key and P-key must be matched to the sequencing platform (examples below are for Ion Torrent) and can be adapted as necessary.

<table>
<thead>
<tr>
<th>A-key</th>
<th>Forward</th>
<th>FID (8)</th>
<th>Forward handle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward 5’-CCATCTCATCCCTGCGTGTCTCCGACTCAG + TG + Sample ID + GAAGTTCCAGCCAGCGTC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-key</td>
<td>RID (8)</td>
<td>Reverse handle</td>
<td></td>
</tr>
<tr>
<td>Reverse 5’-CCATCTCATCCCTGCGTGTCTCCGACTCAG + TG + Sample ID + CTGTGACTATGTGAGGCTTTC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Examples of primer sequences for Ion Torrent sequencing (color-coding as above):

<table>
<thead>
<tr>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCATCTCATCCCTGCGTGTCTCCGACTCAG + TG + Sample ID + GAAGTTCCAGCCAGCGTC-3’</td>
<td>CTGTGACTATGTGAGGCTTTC-5’</td>
</tr>
<tr>
<td>CCATCTCATCCCTGCGTGTCTCCGACTCAG + TG + Sample ID + CTGTGACTATGTGAGGCTTTC-3’</td>
<td>CCATCTCATCCCTGCGTGTCTCCGACTCAG + TG + Sample ID + GAAGTTCCAGCCAGCGTC-3’</td>
</tr>
</tbody>
</table>