dCODE Dextramer® (HiT) Package Insert

Products

dCODE Dextramer® (HiT) (Gold/Explore), cat# WBxxxxxdHG / UWBxxxxxHEP
CD1d dCODE Dextramer® (HiT) (Gold/Explore), cat# XDxxxxxdHG / YDxxxxxdHG
MR1 dCODE Dextramer® (HiT) (Gold/Explore), cat# ZAxxxxxdHG
Collectively denominated dCODE® (HiT) reagents

Recommended use

Profiling and quantitation of antigen-specific T cells, NKT, or MAIT cells in cell samples[1,2,3].

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

Materials Provided

dCODE® (HiT) reagents consisting of a dextran polymer backbone carrying multiple MHC, CD1d, or MR1-antigen complexes, a corresponding unique DNA Barcode oligo, and R-phycoerythrin (PE) for sorting of dCODE® (HiT) positive cells.

- dCODE® (HiT) reagents are provided at a concentration of 160 nM in PBS buffer, containing 1% bovine serum albumin (BSA) and 15 mM NaN_3, pH 7.2.
- 2 µL (one test) is recommended for staining of 1-3 x 10^6 PBMCs.
- Each dCODE® (HiT) reagent is uniquely identified by its allele / Peptide / DNA Barcode.

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'-CTGTGACTATGTGAGGCTTTCXXXXXXXXXXXXXXXXXXNNNNNNNNNNNNNNNNNNGACGCTGGCTGGAACTTC-3'

Sizes

dCODE® (HiT) - Gold: Single reagents of 25 tests (50 µL), 50 tests (100 µL) or 150 tests (300 µL) each.

dCODE® (HiT) - Explore: Reagent Panels of 16, 32, 48, 64, 80, or 96 for 10 tests (20 µL), 25 tests (50 µL) or 50 tests (100 µL) each.

Storage

dCODE® (HiT) reagents should be stored at 2-8°C in the dark – the plastic vial only partially protects the reagents against light.

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.

References

1. Bentzen, A. et al., Large-scale detection of antigen-specific T cells using peptide-MHC-I multimers labeled with DNA barcodes. Nat. Biotechnol 2016. Doi: 10.1038/nbt.3662
2. Bentzen, A. et al., Next-generation detection of antigen-responsive T cells using DNA barcode-labeled peptide-major histocompatibility complex I multimers. European Journal of Immunology, 2016. Doi: 10.1002/eji.201670200
3. Lyngaa, R. B et al., High-throughput discovery of T cell epitopes in type 1 diabetes using DNA barcode labelled peptide-MHC multimers. Eu Jour. of Immunol., 2016. DOI: 10.1038/nbt.3662

Patents

The dCODE® technology is disclosed in granted and pending patents within the WO 2015/185067 and WO 2015/188839 patent families including US11402373, US11585806, EP3152232, AU2015271324, AU2019264685, SG11201610177U and JP6956632.

Symbols

See www.immudex.com/symbols for explanation of symbols.

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Materials Required (Not Provided)

Stain buffer: PBS, pH 7.4 cont. 1-5% serum and 0.1 g/L Herring sperm DNA
Wash buffer: PBS, pH 7.4 cont. 1-5% serum
Sorting buffer: PBS, pH 7.4 cont. 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 see Appendix A
qPCR reaction components
QIAquick® PCR Purification Kit

Procedure

1. Prepare PBMC sample and resuspend 1-3 x 10^6 PBMC in 50-100 µL stain buffer.
2. Centrifuge dCODE® (HiT) at 10,000 x g for 1 min.
3. Preparation of dCODE® (HiT) reagent pool:
   a. Add 0.2 µL 100 μM d-Biotin per dCODE® (HiT) specificity into an empty tube
   b. Add 2 µL of each dCODE® (HiT) specificity and mix
4. From this mixture, take 1 µL and dilute with 99 µL wash buffer. Save this for qPCR amplification as input sample (step 13)
5. Add the pool of dCODE® (HiT) reagents to the cell sample and mix thoroughly
6. Incubate in the dark at room temperature:
   a. MHC I, MR1 or CD1d dCODE Dextramer® (HiT) pool: 10 min. incubation[A]
   b. MHC II dCODE Dextramer® (HiT): 30 min. incubation[A]
   c. dCODE® (HiT) pool comprized of a. and b.: 30 min. incubation[A]
1. Add relevant antibodies in the volume/concentration recommended by provider. Incubate at room temperature in the dark for 20 min.
7. 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 another 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
8. Resuspend cells in adequate volume of wash buffer
9. Proceed to FACS following the guidelines and practices of your sorting facility
10. Make a two-way sort by sorting the dCODE® (HiT)-PE-positive population and the dCODE® (HiT) - PE-negative population separately
11. Collect sorted cells directly into tubes containing 500 µL sorting buffer.
12. Centrifuge the sorted cell samples at 300 x g for 10 min. Add 1 mL pure PBS and spin down for an additional 5 min. at 300 x g. Discard

supernatant and resuspend cell pellet in 40 µL PBS
(Optional: Store at -20°C for up to 72h or proceed to the next step)

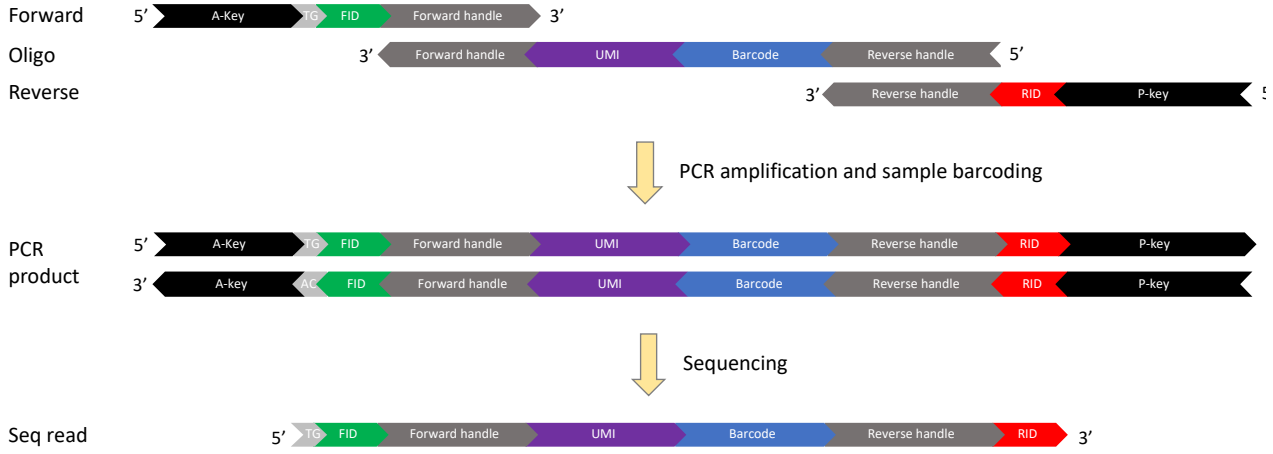
13. For each cell sample (dCODE® (HiT)-PE-positive and dCODE ® (HiT)-PE-negative), use 20 µL cell suspension to prepare a 40 µL final PCR reaction volume. For dCODE ® (HiT) input mixture, use 1 µL of the diluted sample as template in a 40 µL total PCR reaction volume
14. Purify DNA from each PCR reaction (dCODE ®-PE-positive, dCODE®-PE-negative, and input mixture) using QIAquick® PCR Purification Kit following the manufacturer's instructions. Elute each sample in 20 µL water
15. To prepare the final sample for sequencing, mix 10 µL of each of the purified dCODE®-PE-positive and dCODE®-PE-negative PCR products. Add just 1 µL PCR product from the input mixture. Remaining purified PCR products can be stored at -20°C as backup
16. Send sample for sequencing.

Data Analysis

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

Sequencing reads from the dCODE® (HiT) input mixture serve to validate the presence of all dCODE® (HiT) reagents.

Appendix DNA Oligo amplification and Primer design



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.

A-key FID (8) Forward handle
Forward 5'-CCATCTCATCCCTGCGTGTCTCCGACTCAG + TG + Sample ID + GAAGTTCCAGCCAGCGTC-3'

P-key RID (8) Reverse handle
Reverse 5'-CCTCTCTATGGGCAGTCGGTGAT + Sample ID + CTGTGACTATGTGAGGCTTTC-3'

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

Forward Primer	**Reverse Primer**
CCATCTCATCCCTGCGTGTCTCCGACTCAG TGCTGGGGTGGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATATTGCGCCC TGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAG TGCTCCACACGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATGACCCGTAC TGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAG TGCTTACCTGGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATTGGCGTACC TGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAG TGTGGCAGCAGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATTTATATGTC TGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAG TGTGAGTAACGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATTGTTCTGCC TGTGACTATGTGAGGCTTTC