dCODE Dextramer® (HiT)

dCODE Dextramer® (HiT) - Gold **Cat. No. dC01, dC02, dC03**
dCODE Dextramer® (HiT) - Explore **Cat. No. dCE01**

Recommended use

Profiling and quantitation of antigen-specific T cells in cell samples[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-phycoerythrin (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'-CTGTGACTATGTGAGGCTTTCXXXXXXXXXXXXXXXXXXNNNNNNNNNNNNNNNNNNGACGCTGGCTGGAACTTC-3'

dCODE Dextramer (HiT) 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/test is recommended for staining of 1-3 x 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 µl), 50 tests (100 µl) or 150 tests (300 µl) each.

dCODE Dextramer (HiT) - Explore: Panels of 16, 32, 48, 64, 80, or 96 dCODE Dextramer (HiT) 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 (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.

As with any product derived from biological sources, proper handling procedures should be used.
For professional users.

References

1. Bentzen, A., Marquard, A., Lyngaa, R. et al. Large-scale detection of antigen-specific T cells using peptide-MHC-I multimers labeled with DNA barcodes. Nat Biotechnol 34, 1037–1045 (2016).

2. Bentzen, A. K., Marquard, A. M., Lyngaa, R. B., Saini, S. K., Andersen, S. R., Donia, M., Svane, I. M., thor Straten, P., Szallasi, Z. I., Jakobsen, S. N., Eklund, A. C., & Hadrup, S. R. (2016). Next-generation detection of antigen-responsive T cells using DNA barcode-labeled peptide-major histocompatibility complex I multimers. European Journal of Immunology, 46(S1), 8-8. [1831]. https://doi.org/10.1002/eji.201670200

3. Lyngaa, R. B., Bentzen, A. K., Overgaard, A. J., Piciot, F., Størling, J., & Hadrup, S. R. (2016). High-throughput discovery of T cell epitopes in type 1 diabetes using DNA barcode labelledpeptide-MHC multimers. European Journal of Immunology, 46(Suppl. 1), 931-931.

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.

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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 10^6 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 as input sample (step 13).
4. Add the pool of dCODE Dextramer reagents to the cell sample and mix thoroughly.
5. Incubate at room temperature for 20 min.
6. Add relevant antibodies in the volume/concentration recommended by provider. Incubate 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 $CD8^+$Dextramer-PE-positive population and the $CD8^+$Dextramer-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 (Dextramer-PE-positive and Dextramer-PE-negative), use 20 µl cell suspension to prepare a 40 µl final PCR reaction volume. For Dextramer 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 (Dextramer-PE-positive, Dextramer-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 Dextramer-PE-positive and Dextramer-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.

Analysis of data

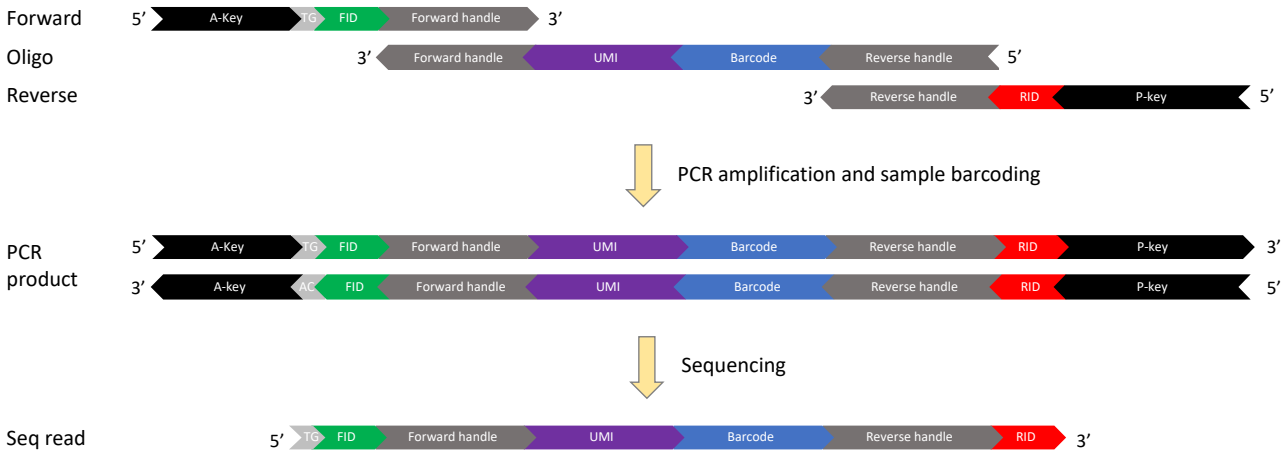
Demultiplex the sequencing data according to sample (primer barcodes) and Dextramer reagents (oligo barcodes). Calculate the apparent enrichment (AE) for each reagent by dividing its read count in the Dextramer-PE-positive sample with the corresponding read count in the Dextramer-PE-negative sample.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



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.

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

Forward Primer	Reverse Primer
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCTGGGGTGGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATATTGCGCCCTGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCTCCACACGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATGACCCGTACTGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGCTTACCTGGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATTGGCGTACCTGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTGGCAGCAGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATTTATATGTCTGTGACTATGTGAGGCTTTC
CCATCTCATCCCTGCGTGTCTCCGACTCAGTGTGAGTAACGAAGTTCCAGCCAGCGTC	CCTCTCTATGGGCAGTCGGTGATTGTTCTGCCTGTGACTATGTGAGGCTTTC