dCODE® Klickmer

dCODE® Klickmer (10x compatible) **Cat. No. dCXC**
dCODE® Klickmer (HiT) **Cat. No. dCC**

Recommended use

Customization of dCODE Klickmer with ligand for profiling and quantitation of specific cell subsets based on receptor recognition of the ligand.

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

Reagents provided

dCODE Klickmer consists of a dextran polymer backbone carrying an average of 20 acceptor sites for biotinylated ligands, a unique DNA Barcode oligo and R-phycoerythrin (PE) for sorting of dCODE Klickmer positive cells.

The actual binding capacity will depend on the physical properties of the biotinylated ligand.

Each dCODE Klickmer is uniquely identified by its DNA Barcode number: (10x compatible) fBCNNNN or (HiT) HiTNNNN.

For dCODE Klickmer (10x compatible), the unique DNA barcode oligo comprises:

- Primer sequence compatible with Illumina® Sequencers (Nextera pR2)
- Split Unique Molecule Identifier (UMI) sequences
- DNA Barcode sequence that specifies the ligand
- Capture sequence for 10x Chromium single cell immune profiling solution

Nextera pR2 UMI (10) DNA Barcode (15) UMI (9) Capture seq

5′-CGGAGATGTGTATAAGAGACAGNNNNNNNNNNXXXXXXXXXXXXXXXNNNNNNNNNCCCATATAAGAAA-3′

For dCODE Klickmer (HiT), 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 ligand

Reverse handle DNA Barcode (18) UMI (18) Forward handle

5′-CTGTGACTATGTGAGGCTTTCXXXXXXXXXXXXXXXXXXNNNNNNNNNNNNNNNNNNGACGCTGGCTGGAACTTC-3′

dCODE Klickmer is provided at a concentration of 2.7 x 10^{-7} M in PBS buffer containing 1% bovine serum albumin (BSA) and 15 mM NaN_3, pH 7.2.

Sizes

dCODE Klickmer is provided as single reagents in volumes 30 µl, 60 µl and 180 µl or in panels of 16, 32, 48, 64, 80, and 96 reagents in volumes of 30 µl and 60 µl.

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.

Symbols

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

Technical support

E-mail: customer@immudex.com

Telephone: +45 3110 9292 (Denmark)

Manufacturer

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

dCODE® Klickmer

Assembling Protocol

Materials required (not provided)

Biotinylated ligand to be assembled with the dCODE Klickmer should preferably:

- be mono-biotinylated,
- prepared in aqueous buffer pH 7.0-7.5,
- have a biotinylation level > 75%.
- be free of excess biotin.

Dilution buffer: PBS containing 1% BSA, pH 7.

Assembling procedure

It is recommended to titrate the amount of biotinylated ligand to be assembled with dCODE Klickmer (the valency) to reach the desired sensitivity of the final assembled reagent by testing at least 3 different ratios of biotinylated ligand per dCODE Klickmer. Too low valency may result in too low avidity to detect the target, too high valency may generate unwanted background staining.

1. Calculate the amount of biotinylated ligand needed to produce the desired volume and stoichiometry between dCODE Klickmer and biotinylated ligand. You can find a calculation example in procedural notes.

$$\text{Your biotinylated ligand volume (l)} = \frac{\text{dCODE Klickmer volume (l)} * \text{dCODE Klickmer concentration (mol/l)} * \text{Desired number of ligands per dextran}}{\text{Your biotinylated ligand concentration (mol/l)}}$$

2. Pipette the calculated amount of biotinylated ligand into a dark reaction tube.
3. Pipette dCODE Klickmer to the biotinylated ligand and mix immediately by pipetting (avoid the formation of foam).
4. Incubate at room temperature for 30 minutes, in the dark.
5. Optionally, add Dilution buffer to reach the desired concentration.

Procedural notes

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

As a guideline, our optimized dCODE Klickmer for flow cytometry, are 160 nM, where 2 µl is used to stain up to 1 million lymphocytes.

For staining protocols using MHC dCODE Klickmer reagents, please see www.immudex.com

Example to determinate the volume of biotinylated ligand to pipette to assemble 20 µl (20×10^{-6} l) of dCODE Klickmer reagent (270×10^{-9} mol/l) with 5 ligands per dextran:

$$\text{Your biotinylated ligand volume (l)} = \frac{(20 \times 10^{-6}) * (270 \times 10^{-9}) * (5)}{\text{Your biotinylated ligand concentration (mol/l)}}$$