dCODE Klickmer®

dCODE Klickmer (RiO)	**Cat. No. dRK/dRKP**
dCODE Klickmer (10x)	**Cat. No. dXK/dXKP**
dCODE Klickmer (HiT)	**Cat. No. dHK/dHKP**

Recommended use

dCODE Klickmer is a DNA barcoded reagent carrying multiple acceptor sites for mono-biotinylated ligands. dCODE Klickmer is intended for making customized reagents for profiling and quantitation of specific cell subsets based on receptor recognition of the ligand attached. The DNA barcode allows identification of target population using Next Generation Sequencing and combination with single-cell multi-omics analysis.

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 mono-biotinylated ligands, a unique DNA Barcode oligo and R-phycoerythrin (PE) for enrichment of dCODE Klickmer positive cells.

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

Each dCODE Klickmer is uniquely identified by its DNA Barcode number:

- dCODE Klickmer (RiO) – ADEXNNNN
- dCODE Klickmer (10x) – fBCNNNN
- dCODE Klickmer (HiT) – HiTNNNN

For dCODE Klickmer (RiO), 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 (10x), 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 compatible with the 10x Chromium single cell immune profiling workflow

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

5’-
CGGAGATGTGTATAAGAGACAGNNNNNNNNNNXXXXXXXXXXXXXXXNNNNNNNNNNCCCATATAAGAAA-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’-
CTGTGACTATGTGAGGCTTTCXXXXXXXXXXXXXXXXXXNNNNNNNNNNNNNNNNGACGCTGGCTGGAACTTC-3’

dCODE Klickmer is provided at a concentration of 270 nM 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.

Acceptor sites

The average number of biotin acceptor sites per dCODE Klickmer/PE is ~20.

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.

Patents

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

Symbols & Safety Documentation

See www.immudex.com/symbols

Technical support

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Manufacturer

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

dCODE Klickmer – Assembly Protocol

Materials required (not provided)

Mono-biotinylated ligand to be assembled with dCODE Klickmer must be:

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

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

Procedure

dCODE Klickmer must be assembled freshly for each experiment.

During preliminary assessment, it is recommended to test at least 3 different ratios of mono-biotinylated ligand per dCODE Klickmer (low, medium, and high loading capacity) to optimize the reagent valency for the specific application. Too low valency may result in too low avidity to detect the target while too high valency may generate unwanted background staining. *For recommendations for negative controls, see Procedural Notes.*

1. Calculate the volume of assembled dCODE Klickmer required for the experiment. *For flow cytometry experiments, see Procedural Notes for calculation example.*
2. Calculate the volume of mono-biotinylated ligand required to produce the desired volume of assembled dCODE Klickmer using the equation below. *See Appendix 1 for calculation example.*

$$\text{Your mono-biotinylated ligand volume (µL)} = \frac{\text{Stock dCODE Klickmer volume (µL)} * \text{Stock dCODE Klickmer concentration (nM)} * \text{Number of ligands per dextran}}{\text{Your biotinylated ligand concentration (nM)}}$$

3. In the dark, add the calculated amount of mono-biotinylated ligand into a light protected reaction tube.
4. Add dCODE Klickmer to the mono-biotinylated ligand and mix immediately by pipetting (avoid the formation of bubbles).
5. Incubate at room temperature in the dark, for 30 min.
6. Add dilution buffer to reach the desired concentration. *Reagents need to be pre-diluted for enrichment. See Procedural notes.*
7. Proceed to profiling and quantitation.

Staining Procedures & Sequencing Workflows

Please note that the staining and sequencing workflows will require some optimization to accommodate your composition of ligand on dCODE Klickmer®

1. For dCODE Klickmer® (HiT): See www.immudex.com/Protocols/HiT
2. For dCODE Klickmer® (RiO): See www.immudex.com/Protocols/RiO
3. For dCODE Klickmer® (10x): See www.immudex.com/Protocols/10x

Procedural notes

1. *To use dCODE Klickmer® reagents as negative control,* you could load onto the dCODE Klickmer® molecule a non-relevant ligand like your ligand of interest (same size, same chemical properties, for instance), but that does not bind the cells you are attempting to identify. Please note that an empty dCODE Klickmer® molecule is not an appropriate negative control.
2. Protocol step 6: Dilute assembled dCODE Klickmer in aqueous buffer to a final concentration of 160 nM before staining cells prior to staining.
3. For using dCODE Klickmer reagents to stain prior to enrichment, see the table below to see how the starting volume of Klickmer correlates in test sizes:

Volume stock dCODE Klickmer (270 nM)	Total volume of assembled diluted dCODE Klickmer (160 nM)	Number of tests*
6 µL	10 µL	5
12 µL	20 µL	10
30 µL	50 µL	25
59 µL	100 µL	50
119 µL	200 µL	100
593 µL	1000 µL	500
1185 µL	2000 µL	1000

**Each test of 2 µL (160 nM) is enough to stain 1-3 million PBMCs or 100-300 thousand enriched clonal cells*

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

Appendix 1

Calculation Example

Example to calculate the volume of mono-biotinylated ligand to assemble 10 tests of dCODE Klickmer with n mono-biotinylated ligands per dextran and a mono-biotinylated ligand concentration of (6000 nM):

$$\text{Your mono-biotinylated ligand volume (µL)} = \frac{(11.9\ \mu L) * (270\ nM) * (\text{n mono-biotinylated ligands})}{(6000\ nM)}$$

Table 1. dCODE Klickmer/PE Assembly (biotin acceptor sites ~20)

Ex.	dCODE Klickmer vol. (µL)	dCODE Klickmer conc. (nM)	n ligands	Ligand conc. (nM)	Ligand vol. (µL)	Dilution buffer (µL)	Total volume (µL)
1	11.9	270	5	6000	2.7	5.5	20
2	11.9	270	10	6000	5.4	2.8	20
3	11.9	270	15	6000	8	0.2	20