Recommended use	Customization of Klickmer® with any mono-biotinylated 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	Klickmer® consists of a fluorescent (PE, APC, FITC) dextran backbone, carrying a defined number of acceptor sites for mono-biotinylated ligands. Klickmer® can also be provided without fluorochrome (NONE). The actual loading capacity will depend on the physical properties of the mono-biotinylated molecule. Each Klickmer® is uniquely identified by its fluorophore, e.g., Klickmer® / PE. Klickmer® is provided at a concentration of 160 nM in PBS buffer, containing 1% bovine serum albumin (BSA) and 15 mM NaN_3, pH 7.2.
Sizes	60 µL, 200 µL, 1 mL and 2 mL
Storage	Store in the dark at 2-8°C.
Acceptor sites	The average number of biotin acceptor sites per Klickmer is dependent on the fluorophore label; Klickmer® / APC ~ 10 acceptor sites, Klickmer® / PE ~ 20 acceptor sites, Klickmer® / FITC ~ 40 acceptor sites, Klickmer® / NONE ~ 40 acceptor sites
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.
Symbols & Safety Documentation	See www.immudex.com/symbols
Technical support	E-mail: customer@immudex.com Telephone: +45 3110 9292 (Denmark). +1 (703) 766 4688 (US)
Manufacturer	Immudex, Bredevej 2A, DK-2830 Virum, Denmark

Klickmer® – Assembly Protocol

Materials required (not provided)

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

Procedure

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 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.

1. Calculate the volume of assembled 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 Klickmer® using the equation below. *See Appendix 1 for calculation example.*

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

3. In the dark, add the calculated amount of mono-biotinylated ligand into a light protected reaction tube.
4. Add 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 needs to be pre-diluted for flow cytometry. See Procedural notes.*
7. Proceed to profiling and quantitation.

Procedural notes

1. Protocol step 6: Dilute assembled Klickmer® in aqueous buffer to a final concentration of 32 nM before staining cells for analysis using flow cytometry.
2. To analyze cells in flow cytometry using assembled Klickmer® reagents, follow the protocol "MHC Dextramer® Staining Protocol" (https://www.immudex.com/resources/protocols/)
3. To analyze SARS-CoV-2 Spike-specific B cells by flow cytometry using Spike Klickmer reagents, follow the protocol in the Application Note "Analyze the Response of SARS-CoV-2 Spike-Specific B Cells Using Klickmer® Technology (https://www.immudex.com/resources/educational-material/)
4. For using Klickmer reagents in flow cytometry, see the table below to see how the starting volume of Klickmer® correlates in test sizes:

Volume stock Klickmer® (160 nM)	Total volume of assembled diluted Klickmer® (32 nM)	Number of tests*
10 µL	50 µL	5
20 µL	100 µL	10
50 µL	250 µL	25
100 µL	500 µL	50
200 µL	1000 µL	100
1000 µL	5000 µL	500
2000 µL	10,000 µL	1000

**Each test is enough to stain 1-3 million PBMCs or 100-300 thousand clonal T cells.*

5. Always keep 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 20 µL Klickmer® (160 nM) with n mono-biotinylated ligands per dextran and a mono-biotinylated ligand concentration of (2000 nM):

$$\text{Your mono-biotinylated ligand volume (µL)} = \frac{(20\ \mu L) * (160\ nM) * (n \text{ mono-biotinylated ligands})}{(2000\ nM)}$$

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

Ex.	Klickmer® vol. (µL)	Klickmer® conc. (nM)	n (ligands)	Ligand conc. (nM)	Ligand vol. (µL)
1	20	160	5	2000	8
2	20	160	10	2000	16
3	20	160	15	2000	24

Table 2. Klickmer®/APC Assembly (biotin acceptor sites ~10)

Ex.	Klickmer® vol. (µL)	Klickmer® conc. (nM)	n (ligands)	Ligand conc. (nM)	Ligand vol. (µL)
1	20	160	3	2000	4.8
2	20	160	5	2000	8
3	20	160	7	2000	11.2

Table 3. Klickmer®/FITC Assembly (biotin acceptor sites ~40)

Ex.	Klickmer® vol. (µL)	Klickmer® conc. (nM)	n (ligands)	Ligand conc. (nM)	Ligand vol. (µL)
1	20	160	10	2000	16
2	20	160	20	2000	32
3	20	160	30	2000	48