Recommended use	Customization of 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	Klickmer™ consists of a fluorescent (PE, APC, FITC) dextramer backbone, carrying a defined number of acceptor sites for biotinylated ligands. Klickmer™ can also be provided without fluorochrome (NONE). The actual binding capacity will depend on the physical properties of the 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	200 µL, 1 ml and 2 ml.
Storage	Store in the dark at 2-8°C.
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	See www.immudex.com/symbols
Technical support	E-mail: customer@immudex.com Telephone: +45 3110 9292 (Denmark)
Manufacturer	Immudex, Bredevej 2A, DK-2830 Virum, Denmark

Assembling Protocol

Materials required (not provided)

Biotinylated ligand to be assembled with the 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 Klickmer™ (valency) to reach the desired sensitivity of the final assembled reagent by testing at least 3 different ratios of biotinylated ligand per Klickmer™. 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 amount of biotinylated ligand needed to produce the desired volume and stoichiometry between Klickmer™ and biotinylated ligand. You can find a calculation example in procedural notes.

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

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

Procedural notes

For flow cytometry, it is recommended to pre-dilute Klickmer™ in aqueous buffer to a final concentration of 32 nM. 10 µL of the pre-diluted reagent are used for the staining of 50-100 µL lymphoid cells (up to 1 x 10^6 lymphoid cells).

See staining protocols using Klickmer™ reagents and MHC monomers (www.immudex.com/resources/protocols/)

Example to calculate the volume of biotinylated ligand to assemble 20 µL (20 x 10^{-6} L) Klickmer (160 x 10^{-9} mol/L) with 5 ligands per dextran:

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