CAT. & Size	A1020023S (1,000 tests)	VKEYBIO-01-2024
	A1020023L (10,000 tests)	For Research Use Only
Storage at	-60°C or below	Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET

Streptavidin-LA

Instruction Manual

1. Introduction

KeyTec® TR-FRET Streptavidin-LA is designed for developing the TR-FRET Assay. In the Protein-Protein Interaction assay, one Biotinylated protein binds to the acceptor (KeyTec® TR-FRET Streptavidin- LA*1), and the other protein is labeled (directly or indirectly) with the donor (KeyTec® TR-FRET Eu/Tb*2). When the two proteins interact, the donor molecule is brought into proximity with the acceptor molecule. Excitation of the donor will result in the generation of the TR-FRET signal at 665 nm, proportional to the extent of protein interaction.

*1 KeyTec® TR-FRET LA: TR-FRET Acceptor Molecule

*2 KeyTec® TR-FRET Solar Eu/Tb: TR-FRET Donor Molecule

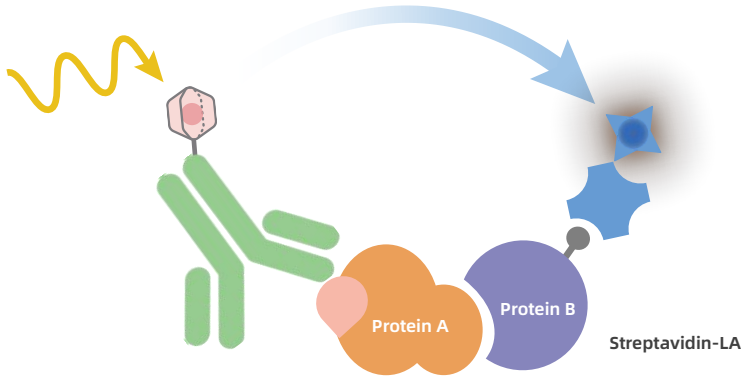

Figure 1. KeyTec® TR-FRET Protein-Protein Interaction assay mode

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2. Components

Components	A1020023S (1,000 tests)	A1020023L (10,000 tests)
KeyTec® TR-FRET Streptavidin-LA (20 µM)	1 vial 50 µL/vial	1 vial 500 µL/vial

KeyTec® Materials Required But Not Supplied	CAT. & Size
KeyTec® TR-FRET Binding Assay Diluent Buffer	A1010001L (200 mL)
KeyTec® TR-FRET Solar Eu Detection Buffer	A1010002L (120 mL)
KeyTec® TR-FRET Solar Tb Detection Buffer	A1010003L (120 mL)
KeyTec® 384-Well White Flat Low-Volume Microplates, PS, Solid, Non-treated, No lid	M2000102N (40 Pcs/Box)
KeyTec® Fluorescent High-Transparency Microplate Top Seals	M1000102N (100 Pcs/Box)

3. Storage Conditions

- Upon receipt, store the reagent below -60 °C.
- Up to 1 years from date of receipt, when stored and handled as recommended.
- When first thaw, aliquot the reagents as needed to avoid multiple freeze-thaw cycles.

4. Assay Procedure

1.1 Assay Format

Assay Format	Total Volume (20 µL[3])
Other assay components	10 µL
KeyTec® TR-FRET Donor (Solar Eu/Tb) working solution (1X)	5 µL
KeyTec® TR-FRET Acceptor (LA/HX) working solution (1X)	5 µL

*[3] The system accommodates 384-well microplates, and assay volumes can be adjusted proportionally to perform in 96-or 1536-well microplates.

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1.2 Reagents Handling

1) Buffers

- KeyTec® TR-FRET Solar Eu/Tb Detection Buffer (A1010002L/A1010003L) has been optimized for maximum performance.
- Use the same buffer to prepare both the donor (Eu/Tb) and the acceptor conjugates.
- KeyTec® TR-FRET Binding Assay Diluent Buffer (A1010001L) is recommended for dilution and preparation of other assay components.
- If using a homemade buffer solution, avoid SDS addition.

2) Conjugates

- Thaw reagents on ice and equilibrate to room temperature before use.
- Prepare working solutions as per the purchased product instructions. The storage solution for KeyTec® TR-FRET Streptavidin-LA is 20 µM; For the first use, it is recommended to use a ratio of 1/4 of the final concentration for Streptavidin-LA (e.g.,10 nM) and Biotinylated protein (e.g.,40 nM) in the assay format. Refer to ***Table-1*** for detailed concentration optimization suggestions and working solution preparation.
- Optimal amounts per well can be further optimized based on different assay format and conditions.

Table-1: KeyTec® TR-FRET Streptavidin–LA concentration optimization suggestions and working solution preparation.

Biotinylated protein Final concentration (example)	SA / Biotin ratio	SA–LA Final concentration	SA–LA Working solution concentration
40 nM	1/1	40 nM	160 nM
	1/2	20 nM	80 nM
	1/4	10 nM	40 nM
	1/8	5 nM	20 nM

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1.3 Data Calculating

- Calculate the ratio of 665 nm/615 nm (TR-FRET Ratio) and the CV for each individual well.

$$\text{TR-FRET Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 615 nm}} \times 10{,}000$$

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