CAT. & Size	A1020024S (1,000 tests)	**VKEYBIO-01-2026**
	A1020024L (10,000 tests)	**For Research Use Only**
Storage at	2-8 ℃	**Not For Diagnostic Or Therapeutic Use**

KeyTec® TR-FRET Streptavidin-HX

Instruction Manual

1. Introduction

KeyTec® TR-FRET Streptavidin-HX 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-HX*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/HX: TR-FRET Acceptor Molecule

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

2. Components

Components	A1020024S (1,000 tests*3)	A1020024L (10,000 tests*3)
KeyTec® TR-FRET **Streptavidin-HX** Lyophilized	1 vial 840 pmoles*4	1 vial 8.4 nmoles*4

*3 Tests refers to the number of experimental wells that can be performed when the total reaction volume is 20 μL and reagents are used at the concentrations recommended in the instruction manual. For more details, please refer to the《Guidelines Manual - KeyTec® TR-FRET Protein Interaction Analysis》.

*4 Each vial contains the total amount of the product. Add ultrapure water to the volume indicated on the product label to reconstitute, resulting in a stock solution with a molar concentration of 20 μM.

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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 2-8 ℃.
- 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. And the reagent must be stored below -60 ℃.

4. Assay Procedure

4.1 Assay Format

Assay Format	Total Volume (20 μL*5)
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

*5 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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4.2 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

- **Before reconstitution :** Please equilibrate the reagent to room temperature and ensure that the stock solution and working solution are prepared according to the instructions for the product you purchased.
- **Reconstitute the KeyTec® TR-FRET Streptavidin-HX, Lyophilized** with ultrapure water **:** Centrifuge the vial at 850 ×g for 1-2 minutes before opening the cap. Add ultrapure water as indicated on the label ; this will yield a stock solution with a molar concentration of 20 μM. Gently tap or invert the vial to ensure thorough dissolution of the lyophilized powder, avoiding vortex shaking. Allow the standard to sit at room temperature for more than 15 minutes to ensure complete dissolution.
- **Prepare working solutions**: The stock solution for KeyTec® TR-FRET Streptavidin-HX is 20 μM; For a total reaction volume of 20 μL per well, add 5 μL of the Streptavidin-HX working solution to each well. For the first use, it is recommended to use a ratio of 1/4 of the final concentration for Streptavidin-HX (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-HX concentration optimization and working solution preparation.

Biotinylated protein Final concentration (example)	SA / Biotin ratio	SA-HX Final concentration	SA-HX 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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4.3 Data Calculating

- Calculate the ratio of 665 nm/620 nm (TR-FRET Ratio) and the CV for each individual well.

$$\text{TR-FRET Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10{,}000$$

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