CAT. & Size	A1020054S (1,000 tests)	VKEYBIO-01-2025
	A1020054L (10,000 tests)	For Research Use Only
Storage at	2-8 °C	Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET

mAb anti-HIS-Bright Eu

Instruction Manual

1. Introduction

KeyTec® TR-FRET Bright Eu is designed for developing the TR-FRET Assay. The anti-HIS antibody is a mouse monoclonal antibody. In the Protein-Protein Interaction assay, one HIS-tagged protein binds to the Donor (KeyTec® TR-FRET mAb anti-HIS-Bright Eu*1), and the other protein is labeled (directly or indirectly) with the Acceptor (KeyTec® TR-FRET LA/HX*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 Bright Eu: TR-FRET Donor Molecule(Europium chelate)

*2 KeyTec® TR-FRET LA/HX: TR-FRETacceptor Molecule

2. Components

Components	A1020054S (1,000 tests*3)	A1020054L (10,000 tests*3)
KeyTec® TR-FRET **mAb anti-HIS-Bright Eu** Lyophilized	1 vial 10 pmoles	1 vial 100 pmoles

*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》.

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KeyTec® Materials Required But Not Supplied	CAT. & Size
KeyTec® TR-FRET Binding Assay Diluent Buffer	A1010001L (200 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 °C.
- Up to 1 years from date of receipt, when stored and handled as recommended.
- Once reconstituted, the reagent must be stored below -60 °C. Aliquot the reagents as needed to avoid multiple freeze-thaw cycles.

4. Assay Procedure

4.1 Assay Format

Assay Format	Total Volume (20 µL*4)
Other assay components	10 µL
KeyTec® TR-FRET Donor (Bright Eu/Tb) working solution (1X)	5 µL
KeyTec® TR-FRET Acceptor (LA/HX) working solution (1X)	5 µL

*4 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

- Use the same buffer to prepare the donor and acceptor (LA/HX) without KF.
- 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
- **.Reconstitute the KeyTec® TR-FRET mAb anti-HIS-Bright Eu, 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 100X stock solution with a molar concentration of 0.2 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 mAb anti-HIS-Bright Eu is 100X; dilute 100 times for a 1X working solution, add 5 µL of working solution to each well (20 µL total reaction volume). For example, mix 50 µL of the stock solution with 4950 µL of KeyTec® TR-FRET Bright Eu Detection Buffer for a 1X working solution.
- Optimal amounts per well can be further optimized based on different assay format and conditions.

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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