

CAT. & Size    A1020007S (1,000 tests)  
                    A1020007L (10,000 tests)

Storage at     2-8 °C

VKEYBIO-01-2024

For Research Use Only

Not For Diagnostic Or Therapeutic Use

## KeyTec® TR-FRET

### mAb anti-GST-LA

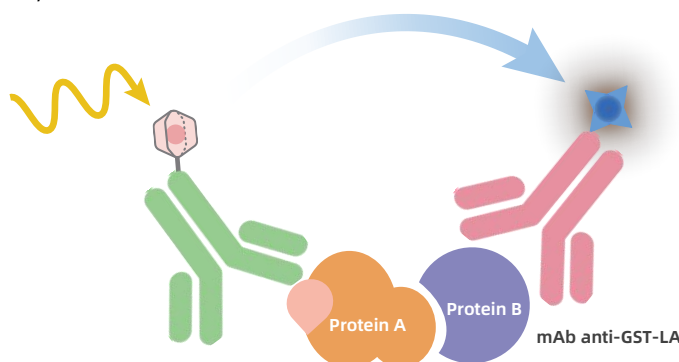
#### Instruction Manual

#### 1. Introduction

**KeyTec® TR-FRET mAb anti-GST-LA** is designed for developing the TR-FRET Assay. The anti-GST antibody is a mouse monoclonal antibody. In the Protein-Protein Interaction assay, one GST-tagged protein binds to the acceptor (**KeyTec® TR-FRET mAb anti-GST-LA**<sup>\*1</sup>), and the other protein is labeled (directly or indirectly) with the donor (**KeyTec® TR-FRET Eu/Tb**<sup>\*2</sup>). 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.

<sup>\*1</sup> KeyTec® TR-FRET LA: TR-FRET Acceptor Molecule

<sup>\*2</sup> KeyTec® TR-FRET Solar Eu/Tb: TR-FRET Donor Molecule



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

## 2. Components

Components	A1020007S (1,000 tests* <sup>3</sup> )	A1020007L (10,000 tests* <sup>3</sup> )
KeyTec® TR-FRET mAb anti-GST-LA, Lyophilized	1 vial	1 vial

\*<sup>3</sup> The tests are sufficient in a 384-well microplate assay format, with 20 µL per well, when using the reagent at the recommended concentration as specified in the manual. For additional details, refer to "Guide\_KeyTec® TR-FRET PPI Assays"

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 °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

### 1.1 Assay Format

Assay Format	Total Volume (20 $\mu$ L <sup>4</sup> )
Other assay components	10 $\mu$ L
KeyTec® TR-FRET Donor (Solar Eu/Tb) working solution (1X)	5 $\mu$ L
KeyTec® TR-FRET Acceptor (LA/HX) working solution (1X)	5 $\mu$ L

\*4 The system accommodates 384-well microplates, and assay volumes can be adjusted proportionally to perform in 96- or 1536-well microplates.

### 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

- ♦ **Reconstitute the KeyTec® TR-FRET mAb anti-GST-LA, Lyophilized, with ddH<sub>2</sub>O:** equilibrate to room temperature, and centrifuge the vial at 6,000 - 10,000 rpm for 10 - 20 seconds or 3,000 rpm for 2 - 3 minutes before use. Add ddH<sub>2</sub>O as indicated on the label ; this will yield a 100X stock solution. 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** as per the purchased product instructions. The stock solution for KeyTec® TR-FRET mAb anti-GST-LA is 100X; dilute 100 times for a 1X working solution. For example, mix 50  $\mu$ L of the storage solution with 4950  $\mu$ L of KeyTec® TR-FRET Solar Eu/Tb Detection Buffer for a 1X working solution.
- ♦ Optimal amounts per well can be further optimized based on different assay format and conditions.

### 1.3 Data Calculating

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

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