

KeyTec® TR-FRET mAb anti-HIS-Green



CAT. & Size A1020043S (1,000 tests)
 A1020043L (10,000 tests)

Storage at -60 °C or below

VKEYBIO-01-2025

For Research Use Only

Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET mAb anti-HIS-Green

Instruction Manual

1. Introduction

KeyTec® TR-FRET mAb anti-HIS-Green 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 acceptor (**KeyTec® TR-FRET mAb anti-HIS-Green**^{*1}), and the other protein is labeled (directly or indirectly) with the donor (**KeyTec® TR-FRET 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 520 nm, proportional to the extent of protein interaction.

^{*1} KeyTec® TR-FRET Solar Green: TR-FRET Acceptor Molecule

^{*2} KeyTec® TR-FRET Tb: TR-FRET Donor Molecule

2. Components

Components	A1020043S (1,000 tests ^{*3})	A1020043L (10,000 tests ^{*3})
KeyTec® TR-FRET mAb anti-HIS-Green (100X)	1 vial 50 µL/vial	1 vial 500 µL/vial

^{*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》.

KeyTec® Materials Required But Not Supplied	CAT. & Size
KeyTec® TR-FRET Binding Assay Diluent Buffer	A1010001L (200 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 -60 °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 (Solar Tb) working solution (1X)	5 µL
KeyTec® TR-FRET Acceptor (Green) 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.

4.2 Reagents Handling

1) Buffers

- ◆ KeyTec® TR-FRET Solar Tb Detection Buffer (A1010003L) has been optimized for maximum performance.
- ◆ Use the same buffer to prepare the donor (Tb).
- ◆ 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.
- ◆ **Prepare working solutions**: The stock solution for KeyTec® TR-FRET mAb anti-HIS-Green 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 Solar Tb 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 520 nm/490 nm (TR-FRET Ratio) and the CV for each individual well.

$$\text{TR-FRET Ratio} = \frac{\text{Signal 520 nm}}{\text{Signal 490 nm}} \times 10,000$$