

CAT. & Size A1020011S (1,000 tests)
 A1020011L (10,000 tests)
Storage at -60°C or below

VKEYBIO-01-2024
For Research Use Only
Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET mAb anti-FLAG-LA Instruction Manual

1. Introduction

KeyTec® TR-FRET mAb anti-FLAG-LA is designed for developing the TR-FRET Assay. The anti-FLAG antibody is a mouse monoclonal antibody. In the Protein-Protein Interaction assay, one FLAG-tagged protein binds to the acceptor (KeyTec® TR-FRET mAb anti-FLAG- 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

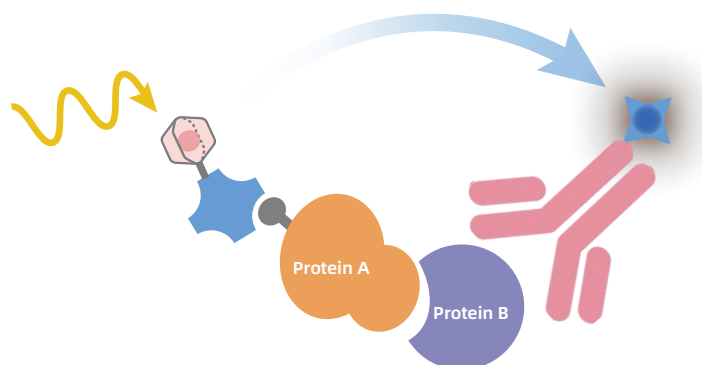


Figure 1. KeyTec® TR-FRET Protein-Protein Interaction assay mode

2. Components

Components	A1020011S (1,000 tests)	A1020011L (10,000 tests)
KeyTec® TR-FRET	1 vial	1 vial
mAb anti-FLAG-LA (100X)	50 µL/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.

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 mAb anti-FLAG-LA is 100X; dilute 100 times for a 1X working solution. For example, mix 50 µL of the storage solution with 4950 µL 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.

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