CAT. & Size	A1020042S (1,000 tests)	VKEYBIO-01-2024
	A1020042L (10,000 tests)	For Research Use Only
Storage at	-60°C or below	Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET

Protein A-LA

Instruction Manual

1. Introduction

KeyTec® TR-FRET Protein A-LA is designed for developing the TR-FRET Assay. Protein A exhibits high affinity towards various species of Ig, particularly IgG. In the Antigen-Antibody Interaction assay, the IgG antibody binds to the acceptor (KeyTec® TR-FRET Protein A-LA*1), and the antigen is labeled (directly or indirectly) with the donor (KeyTec® TR-FRET Eu/Tb*2). When the Antigen-Antibody 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 antigen-antibody interaction.

*1 KeyTec® TR-FRET LA: TR-FRET Acceptor Molecule

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

2. Components

Components	A1020042S (1,000 tests)	A1020042L (10,000 tests)
KeyTec® TR-FRET **Protein A-LA (100X)**	1 vial 50 µL/vial	1 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 Protein A-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.

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