

KeyTec® TR-FRET

pAb anti-Human IgG-Solar Eu



| | | |
|-------------|--------------------------|---------------------------------------|
| CAT. & Size | A1020025S (1,000 tests) | VKEYBIO-01-2025 |
| | A1020025L (10,000 tests) | For Research Use Only |
| Storage at | 2-8 °C | Not For Diagnostic Or Therapeutic Use |

KeyTec® TR-FRET

pAb anti-Human IgG-Solar Eu

Instruction Manual

1. Introduction

KeyTec® TR-FRET pAb anti-Human IgG-Solar Eu is designed for developing the TR-FRET Assay. The anti-Human IgG antibody is a mouse polyclonal antibody. In the Antigen-Antibody Interaction assay, the Human IgG antibody binds to the donor (KeyTec® TR-FRET pAb anti-Human IgG-Solar Eu^{*1}), and the antigen is labeled (directly or indirectly) with the acceptor (KeyTec® TR-FRET LA/HX^{*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 Solar Eu: TR-FRET Donor Molecule

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

2. Components

| Components | A1020025S (1,000 tests ^{*3}) | A1020025L (10,000 tests ^{*3}) |
|--|---|--|
| KeyTec® TR-FRET pAb anti-Human IgG-Solar Eu Lyophilized | 1 vial 20 pmoles ^{*4} | 1 vial 200 pmoles ^{*4} |

*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» .

*⁴ Each vial contains the total amount of the product. Add ultrapure water to the volume indicated on the product label to reconstitute, resulting in a 100X stock solution with a molar concentration of 0.4 µM.

| 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® 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.
- When first thaw, aliquot the reagents as needed to avoid multiple freeze-thaw cycles. And the reagent must be stored below -60 °C.

4. Assay Procedure

4.1 Assay Format

| Assay Format | Total Volume (20 µL ^{*5}) |
|---|-------------------------------------|
| 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 |

^{*5}The assay volume is optimized for 384-well microplates, and can be adjusted proportionally to perform in 96- or 1536-well microplates.

4.2 Reagents Handling

1) Buffers

- KeyTec® TR-FRET Solar Eu Detection Buffer (A1010002L) has been optimized for maximum performance.

- ◆ Use the same buffer to prepare both the donor and the acceptor (LA/HX) conjugates.
- ◆ KeyTec® TR-FRET Binding Assay Diluent Buffer (A1010001L) is recommended for dilution and preparation of other components or samples.
- ◆ If using a homemade buffer solution, avoid SDS and ensure KF 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.
- ◆ **Reconstitute the KeyTec® TR-FRET pAb anti-Human IgG-Solar 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 0.4 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 pAb anti-Human IgG-Solar Eu is 100X; dilute 100 times for a 1X working solution; Add 5 μL of working solution per well (20 μL of total reaction). For example, mix 50 μL of the stock solution with 4950 μL of KeyTec® TR-FRET Solar 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.

$$\text{TR-FRET Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10,000$$