| | | |
|---|---|---|
| CAT. & Size | A1020004S (1,000 tests) | VKEYBIO-01-2024 |
| | A1020004L (10,000 tests) | For Research Use Only |
| Storage at | -60°C or below | Not For Diagnostic Or Therapeutic Use |

# KeyTec® TR-FRET

# mAb anti-HIS-HX

## Instruction Manual

## 1. Introduction

**KeyTec® TR-FRET mAb anti-HIS-HX** 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- HX*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 HX: TR-FRET Acceptor Molecule

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

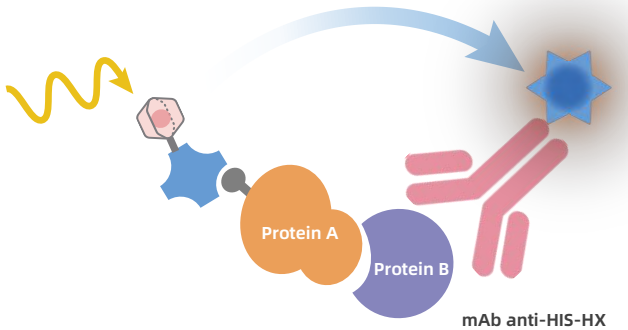


**Figure 1.** KeyTec® TR-FRET Protein-Protein Interaction assay mode



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## 2. Components

| Components | A1020004S (1,000 tests) | A1020004L (10,000 tests) |
|---|---|---|
| KeyTec® TR-FRET **mAb anti-HIS-HX (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.



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## 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-HIS-HX 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.

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



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