

CAT. & Size A1040005S (1,000 tests)  
A1040005L (10,000 tests)

VKEYBIO-01-2024

For Research Use Only

Storage at -60°C or below

Not For Diagnostic Or Therapeutic Use

# KeyTec® TR-FRET Hybridoma screening kit (Human Fc antigen, Mouse IgG) Instruction Manual

## 1. Introduction

The KeyTec® TR-FRET Hybridoma screening kit (Human Fc antigen, Mouse IgG) is designed for the simple and rapid screening of positive mouse hybridoma clones based on antigen-antibody affinity in cell supernatant. It is based on sandwich immunoassay model and utilizes TR-FRET technology, known for its ease of use, homogeneity (no wash), low background, high sensitivity, robustness.

The screening principle is based on TR-FRET technology. The pAb anti hFc-Solar Eu<sup>\*1</sup> binds to the Human Fc-tagged antigen, while the pAb anti-Mouse Fc-LA<sup>\*2</sup> binds to the mouse Fc of IgG antibodies expressed by mouse hybridoma. 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 protein interaction. (Figure 1)

\*1 KeyTec® TR-FRET Solar Eu: TR-FRET Donor Molecule

\*2 KeyTec® TR-FRET LA: TR-FRET Acceptor Molecule

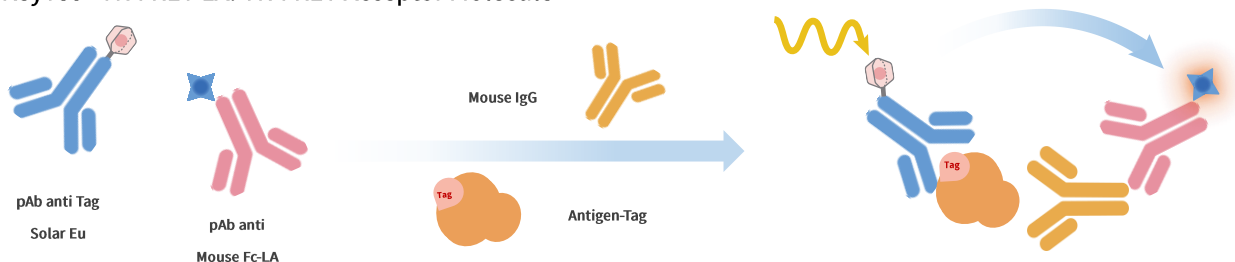


Figure 1. KeyTec® TR-FRET Hybridoma screening kit mode

## 2. Components

Components	Storage	A1040005S (1,000 tests <sup>*3</sup> )	A1040005L (10,000 tests <sup>*3</sup> )
Positive Control (1X)	2-8 °C	1 vial Lyophilized	2 vials Lyophilized
pAb anti hFc-Solar Eu (50X)	≤ -60 °C	1 vial 100 μL/vial	1 vial 1 mL/vial
pAb anti-Mouse Fc-LA (50X)	≤ -60 °C	1 vial 100 μL/vial	1 vial 1 mL/vial
Hybridoma Detection Buffer	2-8 °C	1 bottle 40 mL/bottle	1 bottle 200 mL/bottle

<sup>\*3</sup> The tests are sufficient in a 384-well microplate assay format, with 20 μL per well.

## 3. Storage Conditions

- Upon receipt, store the kit below -60 °C. Kit components remain stable under appropriate storage conditions as recommended.
- Once reconstituted, the standard stock solution may be stored below -60 °C. Aliquot the reagents as needed to avoid multiple freeze-thaw cycles.
- When first thaw, aliquot the components as needed to avoid multiple freeze-thaw cycles
- Volume of standard and antibody aliquots should not be under 10 μL.
- Up to 1 years from date of receipt, when stored and handled as recommended.

## 4. Materials Required But Not Supplied

Materials	Recommended Brand	CAT.
ddH <sub>2</sub> O	Multiple Choices	\
Microplates (KeyTec® 384-Well White Flat Low-Volume Microplates)	VKEY-BIO	M2000102N
KeyTec® Fluorescent High-Transparency Microplate Top Seals	VKEY-BIO	M1000102N
Pipettes	Multiple Choices	\
Microplate Reader With TR-FRET	Multiple Choices	\

## 5. Assay Procedure

### 5.1 Assay Format

Assay Format	Total Volume (20 $\mu\text{L}^{*4}$ )
Sample (Mouse IgG hybridoma Samples)	5 $\mu\text{L}$
Human Fc-Tag antigen	5 $\mu\text{L}$
pAb anti hFc-Solar Eu	5 $\mu\text{L}$
pAb anti-Mouse Fc-LA	5 $\mu\text{L}$

\*4 The system accommodates 384-well microplates, and assay volumes can be adjusted proportionally to perform in 96- or 1536-well microplates.

### 5.2 Reagents Handling

#### 1) Buffers

- Thaw the buffer solution at room temperature and equilibrate before use. The thawed buffer can be stored at 2-8 °C.
- Use the specified buffer to prepare reagents to avoid affecting assay results.

#### 2) Conjugates

- Thaw conjugates on ice, equilibrate to room temperature, and centrifuge before use. Avoid multiple freeze-thaw cycles.
- The stock solution for pAb anti hFc-Solar Eu is 50X; dilute 50 times with Hybridoma Detection Buffer for a 1X working solution (5  $\mu\text{L}$  per well). For example, mix 20  $\mu\text{L}$  of the pAb anti hFc-Solar Eu stock solution with 980  $\mu\text{L}$  of Hybridoma Detection Buffer for a 1 mL 1X working solution.
- The Human Fc-Tag antigen needs to be prepared by the user. Prepare the working solution according to the optimized antigen concentration. Dilute the antigen stock solution with Hybridoma Detection Buffer for a 1X working solution (5  $\mu\text{L}$  per well). For example, if the stock solution for Human Fc-Tag antigen is 2  $\mu\text{M}$  and the optimized working solution concentration is 20 nM, mix 10  $\mu\text{L}$  of the antigen stock solution with 990  $\mu\text{L}$  of Hybridoma Detection Buffer for a 1 mL of 1X working solution.
- The stock solution for pAb anti-Mouse Fc-LA is 50X; dilute 50 times with Hybridoma Detection Buffer for a 1X working solution (5  $\mu\text{L}$  per well). For example, mix 20  $\mu\text{L}$  of the pAb anti-Mouse Fc-LA stock solution with 980  $\mu\text{L}$  of Hybridoma Detection Buffer for a 1 mL 1X working solution.

### 3) Samples

- Generally, mouse hybridoma cell supernatant can be directly tested without dilution. However, if the hybridoma sample is highly concentrated, it can be diluted 2-100 times with the corresponding medium. It's advisable to test and optimize the specific dilution factor in advance for best results.

### 4) Positive Control

- Reconstitute the Positive Control with ddH<sub>2</sub>O:** equilibrate to room temperature, and centrifuge the vial at 6,000-10,000 rpm for 10-20 seconds or 3,000 rpm for 2-3 minutes before use. Add ddH<sub>2</sub>O as indicated on the label. Gently tap or invert the vial to ensure thorough dissolution if the lyophilized powder, avoiding vortex shaking.

## 5.3 Procedure

- Add the reagents to the microplates in turn by following the steps shown in the following table.

	Positive control	Sample
Step 1	10 μL Positive Control	5 μL Human Fc-Tag antigen 5 μL Mouse IgG hybridoma Samples
Step 2	5 μL pAb anti-Mouse Fc-LA	
Step 3	5 μL pAb anti hFc-Solar Eu	
Step 4	Seal the microplate by “KeyTec® Fluorescent High-Transparency Microplate Top Seals” and incubate 1-3 hours at room temperature	
Step 1	(no need to remove the High-Transparency plate sealer) Read on the TR-FRET compatible reader	

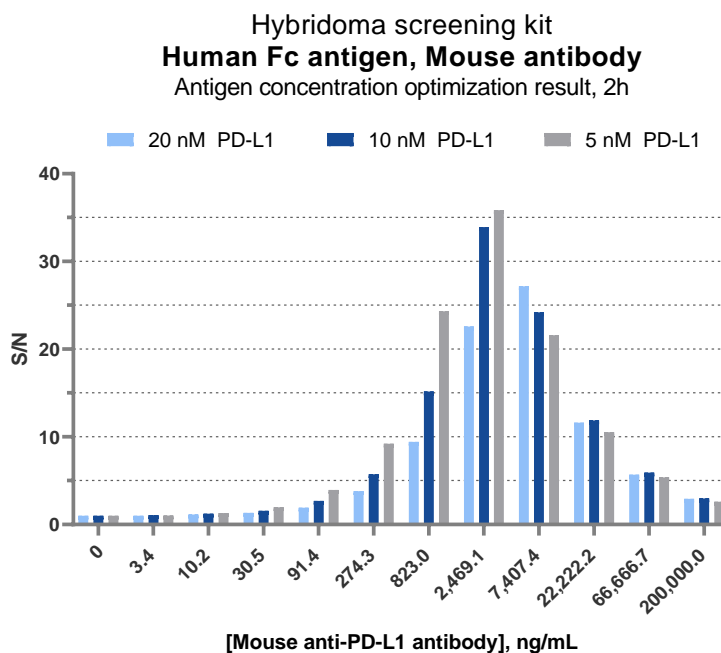
## 5.4 Data Calculating

- Calculate the ratio of 665 nm/615 nm (TR-FRET Ratio) and the CV for each individual well.

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

## 5.5 Performance

- Optimization of Antigen and Antibody Concentration



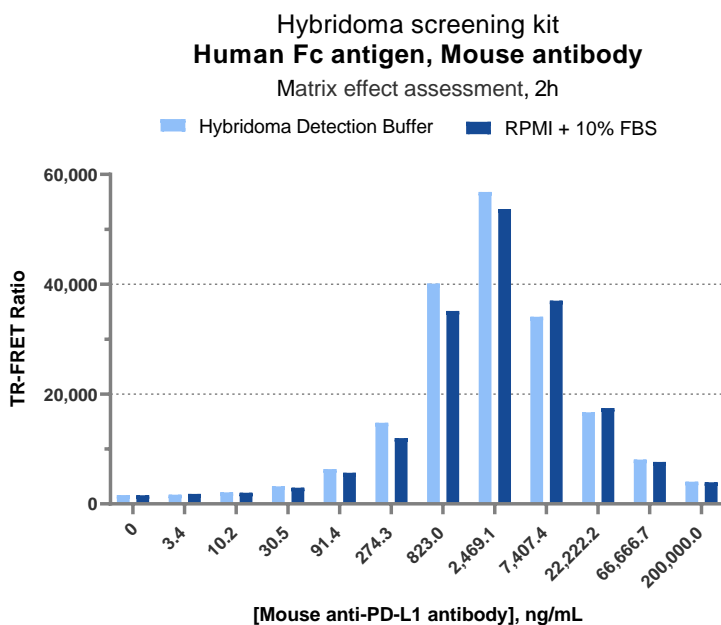
- performance

Range of Antibody concentration: 80 ng/mL – 80  $\mu$ g/mL<sup>5</sup>

Incubation Condition: Incubate at room temperature for 2 hours to overnight

<sup>5</sup> The range of antibody concentration is based on the affinity of the sample(antibody) and the concentration of the antigen.

- Effects of various matrices



◆ Case study

Hybridoma	TR-FRET Ratio					S/B			
	Supernatant	5-fold dilution	25-fold dilution	125-fold dilution	Blank	Supernatant	5-fold dilution	25-fold dilution	125-fold dilution
Clone #1	10,160	20,098	33,886	10,626	1,333	7.6	15.1	25.4	8.0
Clone #2	19,125	28,744	10,491	3,451	1,306	14.6	22.0	8.0	2.6
Clone #3	17,737	29,537	12,196	3,853	1,307	13.6	22.6	9.3	2.9
Clone #4	1,130	1,125	1,133	1,139	1,294	0.9	0.9	0.9	0.9
Clone #5	11,892	20,956	35,404	19,636	1,308	9.1	16.0	27.1	15.0
Clone #6	1,133	1,148	1,120	1,115	1,292	0.9	0.9	0.9	0.9
Clone #7	13,498	27,176	23,113	6,298	1,293	10.4	21.0	17.9	4.9
Clone #8	9,175	20,385	33,543	11,109	1,342	6.8	15.2	25.0	8.3
Clone #9	1,106	1,064	1,134	1,095	1,312	0.8	0.8	0.9	0.8
Clone #10	1,088	1,094	1,114	1,084	1,310	0.8	0.8	0.9	0.8

**Tip:** The data provided above is for reference only. Actual results may vary depended on the performance of the microplate reader used.