CAT. & Size	A1040003S (1,000 tests)	VKEYBIO-01-2024
	A1040003L (10,000 tests)	For Research Use Only
Storage at	-60°C or below	Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET Hybridoma screening kit (His antigen, Mouse IgG)

Instruction Manual

1. Introduction

The KeyTec® TR-FRET Hybridoma screening kit (His 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 mAb anti His-Solar Eu*1 binds to the HIS-tagged antigen, while the pAb anti-Mouse Fc-LA*2 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

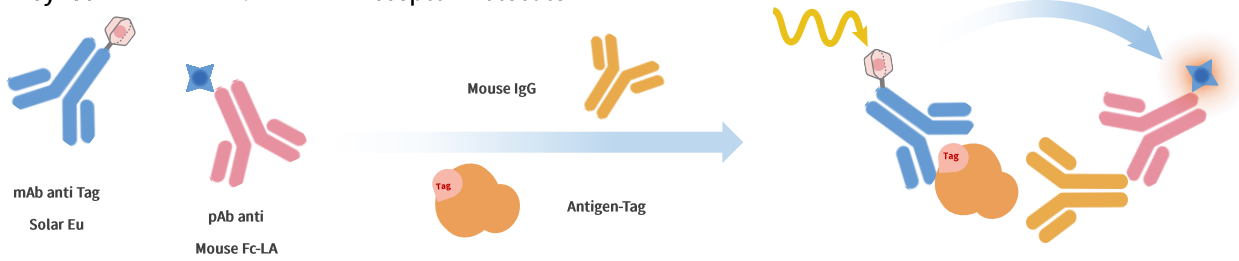

Figure 1. KeyTec® TR-FRET Hybridoma screening kit mode

Shanghai VKEY Biotechnologies Co.,Ltd.
3F, Building 2, 898 Halei Road, Pudong New District, Shanghai.
Website：www.vkeybio.com
Email：technical-support@vkeybio.com

2. Components

Components	Storage	A1040003S (1,000 tests*3)	A1040003L (10,000 tests*3)
Positive Control (1X)	2-8 °C	1 vial Lyophilized	2 vials Lyophilized
mAb anti His-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

*3 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_2O	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	\

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5. Assay Procedure

5.1 Assay Format

Assay Format	Total Volume (20 μL*4)
Sample (Mouse IgG hybridoma Samples)	5 μL
His-Tag antigen	5 μL
mAb anti His-Solar Eu	5 μL
pAb anti-Mouse Fc-LA	5 μL

*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 mAb anti His-Solar Eu is 50X; dilute 50 times with Hybridoma Detection Buffer for a 1X working solution (5 μL per well). For example, mix 20 μL of the mAb anti His-Solar Eu stock solution with 980 μL of Hybridoma Detection Buffer for a 1 mL 1X working solution.
- The His-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 μL per well). For example, if the stock solution for His-Tag antigen is 2 μM and the optimized working solution concentration is 20 nM, mix 10 μL of the antigen stock solution with 990 μL 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 μL per well). For example, mix 20 μL of the pAb anti-Mouse Fc-LA stock solution with 980 μL of Hybridoma Detection Buffer for a 1 mL 1X working solution.

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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_2O:** 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_2O 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 His-Tag antigen 5 µL Mouse IgG hybridoma Samples
Step 2	5 µL pAb anti-Mouse Fc-LA	
Step 3	5 µL mAb anti His-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.

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

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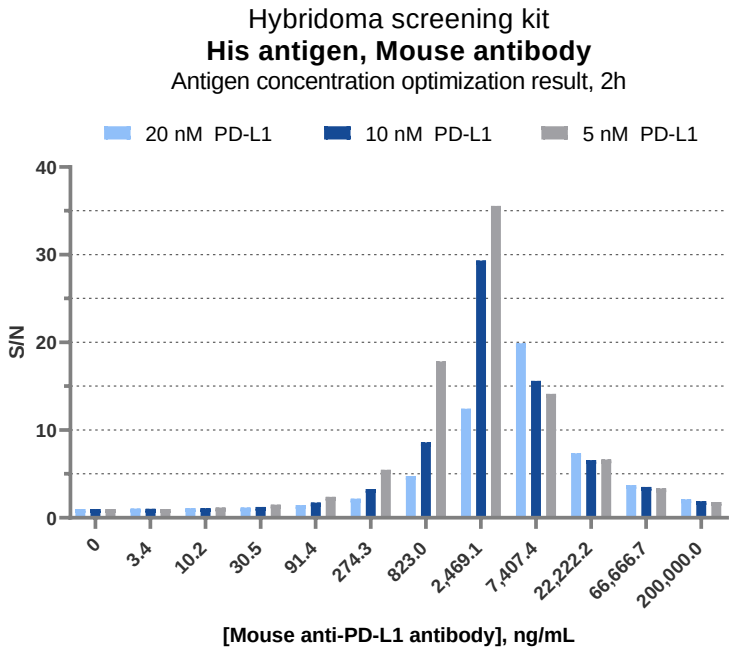
5.5 Performance

- Optimization of Antigen and Antibody Concentration



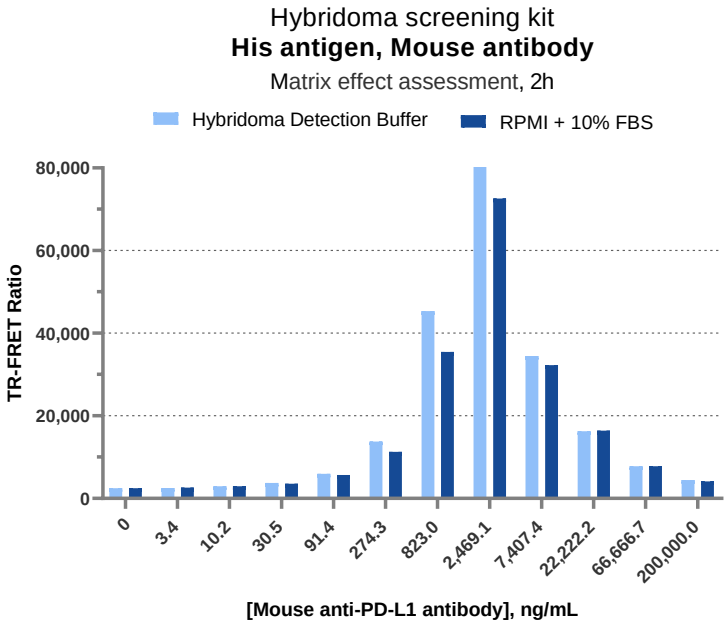
- performance

Range of Antibody concentration: 100 ng/mL – 80 μg/mL[*5]

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

[*5] The range of antibody concentration is based on the affinity of the sample(antibody) and the concentration of the antigen.

- Effects of various matrices



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- Case study

Hybridoma	TR-FRET Ratio					S/B			
Number of samples	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.

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