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

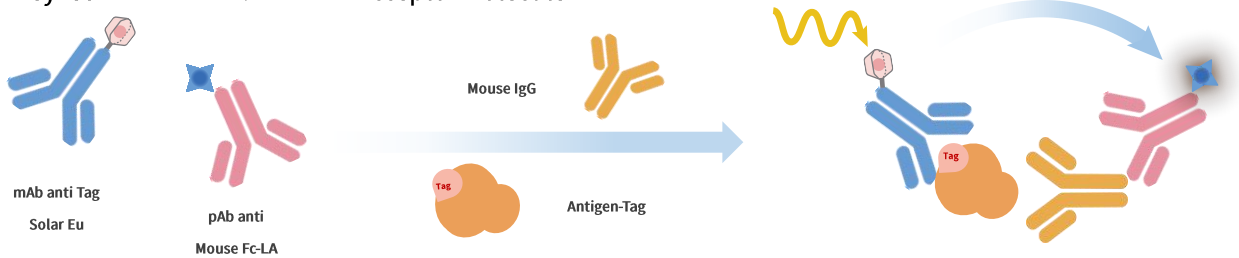


Figure 1. KeyTec® TR-FRET Hybridoma screening kit mode

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	\

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

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.

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

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

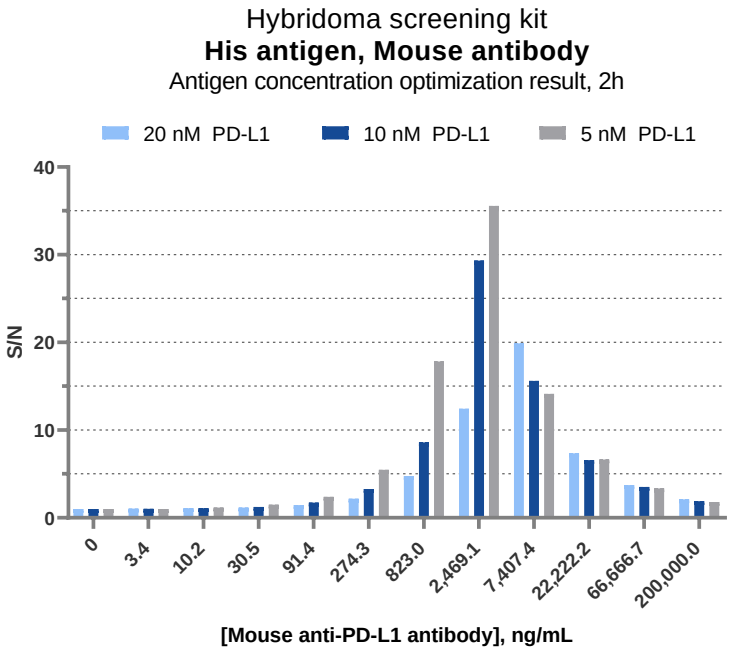
- Calculate the ratio of 665 nm/615 nm (TR-FRET Ratio) and the CV for each individual well.

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

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

5.5 Performance

- Optimization of Antigen and Antibody Concentration



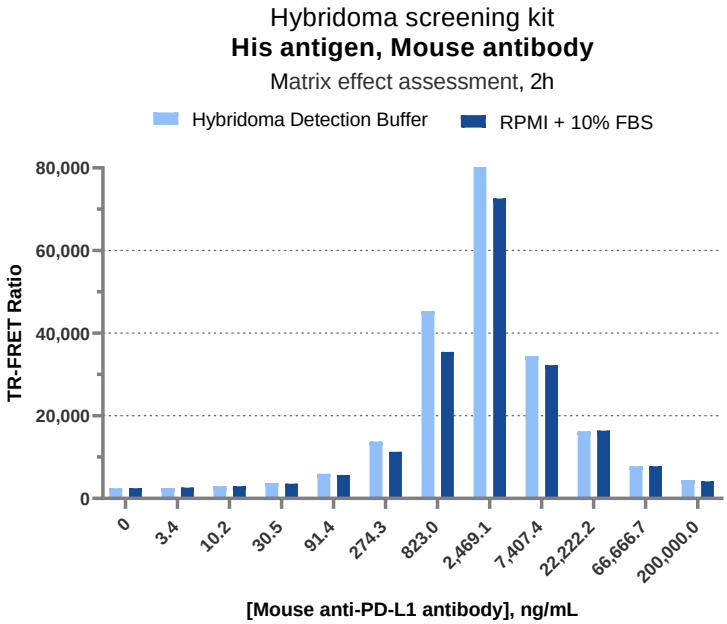
- performance

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



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

- 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.

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