KeyTec® TR-FRET

Hybridoma screening kit (His antigen, Rat IgG)



CAT. & Size A1040007S (1,000 tests) **VKEYBIO-01-2024**

A1040007L (10,000 tests)

For Research Use Only

Email: technical-support@vkeybio.com

-60°C or below Storage at

Not For Diagnostic Or Therapeutic Use

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

Instruction Manual

Introduction 1.

The KeyTec® TR-FRET Hybridoma screening kit (His antigen, Rat IgG) is designed for the simple and rapid screening of positive rat 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 Eu¹ binds to the HIS-tagged antigen, while the pAb anti-Rat Fc-LA*2 binds to the rat Fc of IgG antibodies expressed by rat 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)

^{*2} KeyTec® TR-FRET LA: TR-FRET Acceptor Molecule

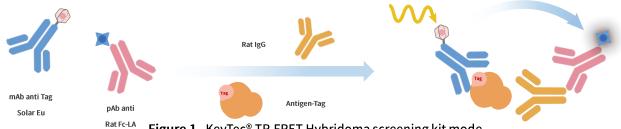


Figure 1. KeyTec® TR-FRET Hybridoma screening kit mode

^{*1} KeyTec® TR-FRET Solar Eu: TR-FRET Donor Molecule



2. Components

Components	Storage	A1040007S (1,000 tests*3)	A1040007L (10,000 tests*3)	
Positive Control (1X)	2-8°C	1 vial	2 vials	
Fositive Control (1x)	2-6 C	Lyophilized	Lyophilized	
mAb anti His-Solar Eu (50X)	≤ -60°C	1 vial	1 vial	
IIIAD aiiti His-Solai Eu (SOA)	≪ -60 C	100 μL/vial	1 mL/vial	
nAh anti Dat Es LA (EOV)	≤ -60°C	1 vial	1 vial	
pAb anti-Rat Fc-LA (50X)	≪ -60 C	100 μL/vial	1 mL/vial	
Uniberial area Data etian Duffer	2-8°C	1 bottle	1 bottle	
Hybridoma Detection Buffer	2-8 C	40 mL/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.
ddH₂O	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	١



5. Assay Procedure

5.1 Assay Format

Assay Format	Total Volume (20 μL*⁴)
Sample (Rat IgG hybridoma Samples)	5 μL
His-Tag antigen	5 μL
mAb anti His-Solar Eu	5 μL
pAb anti-Rat 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 freezethaw 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-Rat 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-Rat Fc-LA stock solution with 980 μL of Hybridoma Detection Buffer for a 1 mL 1X working solution.



3) Samples

• Generally, rat 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 ddH₂O: 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 ddH₂O 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 Rat IgG hybridoma Samples		
Step 2	5 μL pAb anti-Rat 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 2 hours to overnight 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.



5.5 Performance

performance

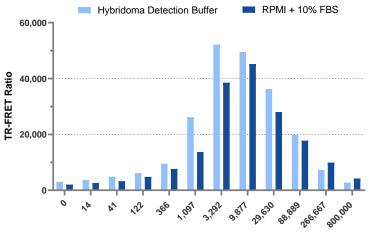
Range of Antibody concentration: 300 ng/mL-300 μg/mL*5

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

Effects of various matrices

Hybridoma screening kit His antigen, Rat antibody

Matrix effect assessment, 2h



[Rat anti-hTNFα antibody], ng/mL

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.

^{&#}x27;5 The range of antibody concentration is based on the affinity of the sample(antibody) and the concentration of the antigen.