CAT. & Size	A1090002S (1,000 tests)	VKEYBIO-01-2024
	A1090002L (10,000 tests)	For Research Use Only
Storage at	-60°C or below	Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET

GDP loaded KRAS[G12D]/RAF1 Binding Assay kit

Instruction Manual

1. Introduction

The KeyTec® TR-FRET GDP loaded KRAS[G12D]/RAF1 Binding Assay kit is designed for measurement of the interaction between KRAS[G12D] and RAF1 proteins. 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, high accuracy.

The detection principle is based on TR-FRET technology. The Tag2-RAF1 protein binds to the donor (KeyTec® TR-FRET mAb anti-HIS-Solar Eu*1), and the Tag1-KRAS[G12D] [GDP loaded] protein binds to the acceptor (mAb anti-Tag1–LA*2). When KRAS[G12D] [GDP loaded] interacts with RAF1, 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. Blocking this interaction with any compound, peptide, or antibody will reduce the TR-FRET signal.

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

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

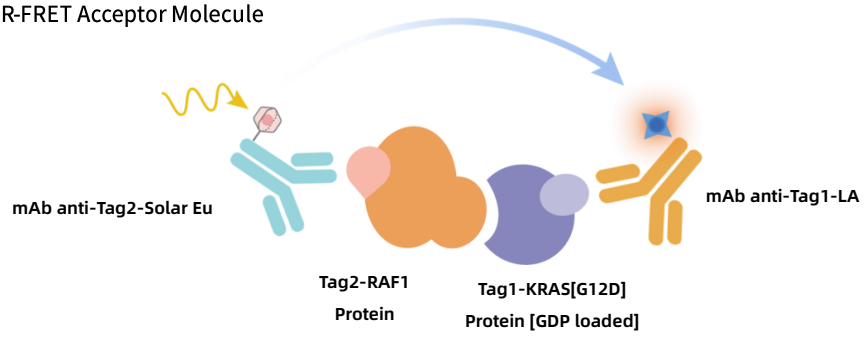

Figure 1. KeyTec® TR-FRET KRAS[G12D]/RAF1 Binding Assay kit mode

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2. Components

Components	Storage	A1090002S (1,000 tests[*3])	A1090002L (10,000 tests[*3])
Tag1-KRAS[G12D] [GDP loaded] (100X)	⩽ -60 °C	1 vial 45 µL/vial	1 vial 410 µL/vial
Tag2-RAF1 (100X)	⩽ -60 °C	1 vial 45 µL/vial	1 vial 410 µL/vial
mAb anti-Tag2–Solar Eu (100X)	⩽ -60 °C	1 vial 50 µL/vial	1 vial 500 µL/vial
mAb anti-Tag1–LA (100X)	⩽ -60 °C	1 vial 50 µL/vial	1 vial 500 µL/vial
GTP (100X，2.5 mM)	⩽ -60 °C	1 vial 45 µL/vial	1 vial 410 µL/vial
Binding Assay Diluent Buffer	2-8 °C	1 bottle 50 mL/vial	1 bottle 200 mL/bottle
Solar Eu Detection Buffer	2-8 °C	1 bottle 30 mL/vial	1 bottle 120 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.
- When first thaw, aliquot the components as needed to avoid multiple freeze-thaw cycles
- Up to 1 years from date of receipt, when stored and handled as recommended.

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4. Materials Required But Not Supplied

Materials	Recommended Brand	CAT.
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

- The table below provides the volume and concentration of each component in the 20μL reaction system.

Assay Format	Add Volume	Stock Concentration	Work Concentration	Final Concentration
Sample	2 μL	Vary with sample	10X	1X
Tag1-KRAS[G12D]	4 μL	100X	1X	\
Tag2-RAF1	4 μL	100X	1X	\
GTP (pre-mixed with RAF1)	\	100X	1X	\
mAb anti-Tag2–Solar Eu	5 μL	100X	1X	\
mAb anti-Tag1–LA	5 μL	100X	1X	\

*4 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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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) Samples

- Dilute the samples using Binding Assay Diluent Buffer. Ensure that if the stock contains DMSO, the final DMSO concentration remains consistent across all reaction systems and remains below 0.5%.

3) Proteins

- Thaw proteins on ice, equilibrate to room temperature, and centrifuge before use. Avoid multiple freeze-thaw cycles.
- The stock solution for Tag1-KRAS[G12D] [GDP loaded] is 100X; dilute 100 times with Binding Assay Diluent Buffer for a 1X working solution (4 µL per well). For example, mix 5 µL of the Tag1-KRAS[G12D] [GDP loaded] stock solution with 495 µL of Binding Assay Diluent Buffer for a 500 µL 1X working solution.
- For ease of operation, Tag2-RAF1 and GTP are premixed in this assay. Dilute the 100X Tag2-RAF1 and 100X GTP stock solutions 100 times with Binding Assay Diluent Buffer for a 1X working solution (4 µL per well). After preparing the premixed working solution, incubate it for 10 minutes before use, ensuring the total incubation time does not exceed 20 minutes. The final GTP concentration is 5 µM in the 20 µL reaction system. For example, mix 5 µL of the Tag2-RAF1 stock solution and 5 µL of the GTP stock solution with 490 µL of Binding Assay Diluent Buffer to prepare a 500 µL 1X working solution. Note that GTP is unstable, so prepare the premixed reagent immediately before use.
- Prepare the Tag1-KRAS[G12D] and the Tag2-RAF1 (GTP premixed) solutions separately. Do not premix these solutions.

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4) Conjugates

- Thaw conjugates on ice, equilibrate to room temperature, and centrifuge before use. Avoid multiple freeze-thaw cycles.
- The stock solution for mAb anti-Tag2–Solar Eu is 100X; dilute 100 times with Solar Eu Detection Buffer for a 1X working solution (5 µL per well). For example, mix 5 µL of the mAb anti-Tag2–Solar Eu stock solution with 495 µL of Solar Eu Detection Buffer for a 500 µL 1X working solution.
- The stock solution for mAb anti-Tag1–LA is 100X; dilute 100 times with Solar Eu Detection Buffer for a 1X working solution (5 µL per well). For example, mix 5 µL of the mAb anti-Tag1–LA stock solution with 495 µL of Solar Eu Detection Buffer for a 500 µL 1X working solution.
- Mix the 1X working solutions of mAb anti-Tag2–Solar Eu and mAb anti-Tag1–LA in a 1:1 ratio for pre-mixed Antibodies[*5].

[*5] It is recommended to use the pre-mixed antibodies for testing to reduce operational steps and minimize deviations introduced by operations.

5.3 Procedure

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

	Negative Control	Positive Control	Sample
Step 1	2 µL Diluent Buffer[*6]	2 µL Diluent Buffer[*6]	2 µL sample
Step 2	4 µL Tag2-RAF1 + GTP (1X)		
Step 3	4 µL Diluent Buffer	4 µL Tag1-KRAS[G12D] (1X)	
Step 4	Incubate 15 mins at room temperature		
Step 5	10 µL pre-mixed Antibodies[*7]		
Step 6	Seal the microplate by “KeyTec® Fluorescent High-Transparency Microplate Top Seals” and incubate 2 hours to overnight at room temperature		
Step 7	(no need to remove the High-Transparency plate sealer) Read on the TR-FRET compatible reader		

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*6 If the sample contains DMSO, the final DMSO concentration remains consistent across Negative and Positive control.

*7 It is recommended to use the pre-mixed antibodies for testing to reduce operational steps and minimize deviations introduced by operations.

- Other Control

Components	Diluent Buffer	mAb anti-Tag2–Solar Eu	Detection Buffer
Solar Eu Control*8	10 µL	5 µL	5 µL
Buffer Control*9	10 µL	\	10 µL

*8 Solar Eu Control: Used to assess the signal of TR-FRET donor at 615 nm (or 620 nm). It's recommended to use this setting for the initial assay setup.

*9 Buffer Control: Used to assess the fluorescence background. It's recommended to use this setting for the initial assay setup.

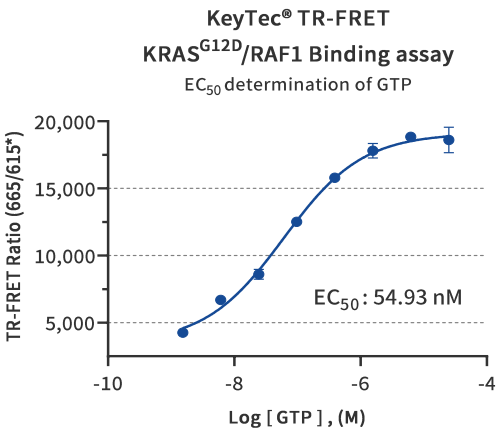
5.4 Data Calculating

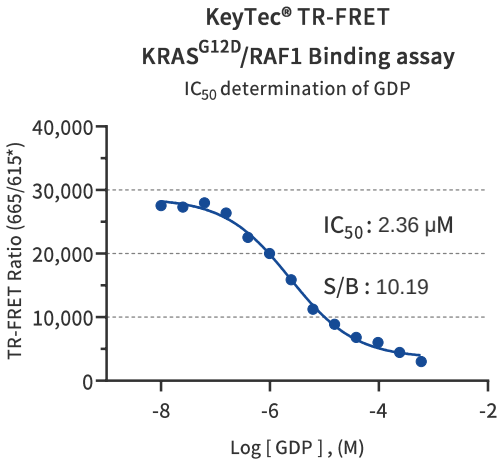
- Calculate the ratio of 665 nm/615 nm (TR-FRET Ratio) and the CV for each individual well.

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

5.5 Performance

- The results of GTP EC50 and GDP IC50





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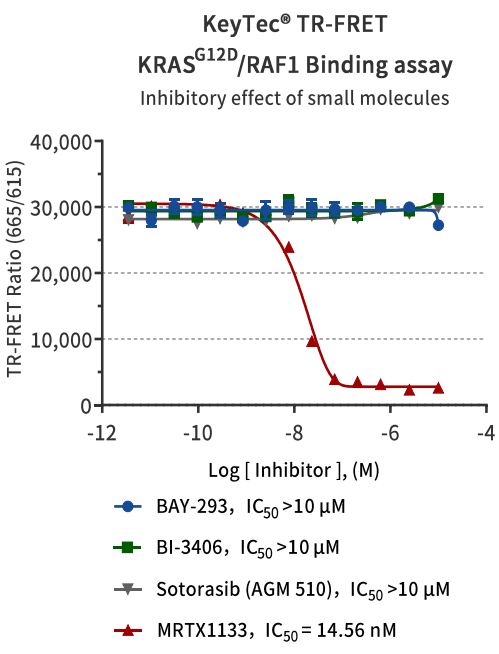
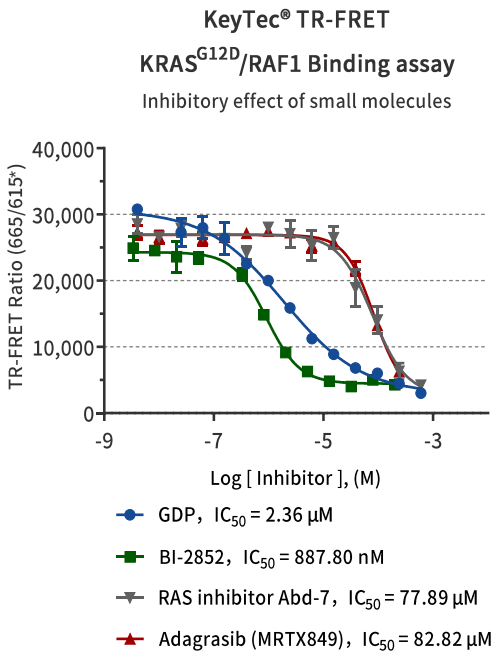
- The inhibitory effects of small molecules





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

6. Related Products

- KeyTec® TR-FRET GDP loaded KRAS[G12D]/SOS1/RAF1 complex Binding Assay kit
- KeyTec® TR-FRET GDP loaded KRAS[G12D]/SOS1 Binding Assay kit
- KeyTec® TR-FRET GDP loaded KRAS[G12V]/SOS1 Binding Assay kit
- KeyTec® TR-FRET GDP loaded KRAS[G13D]/SOS1 Binding Assay kit
- KeyTec® TR-FRET GDP loaded KRAS[G12V]/RAF1 Binding Assay kit
- KeyTec® TR-FRET GDP loaded KRAS[G13D]/RAF1 Binding Assay kit
- KeyTec® TR-FRET GDP loaded KRAS[G12D]/GTP Binding Assay kit
- KeyTec® TR-FRET GDP loaded KRAS[G12V]/GTP Binding Assay kit
- KeyTec® TR-FRET GDP loaded KRAS[G13D]/GTP Binding Assay kit
- Custom assay kits for other RAS-related protein interactions, such as NRAS, HRAS, A-RAF, B-RAF, SOS2 (various mutants)

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