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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
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Description

The KRAS (G12C) Isoform A Coupled Nucleotide Exchange TR-FRET Assay Kit is a TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer) assay kit designed for screening and profiling of KRAS (Kirsten rat sarcoma) (G12C) antagonists/inhibitors. It monitors the binding of an effector protein such as the Ras binding domain of Raf1, (RBD-cRAF), to KRAS(G12C). The KRAS(G12C) Isoform A Coupled Nucleotide Exchange TR-FRET Assay Kit comes in a convenient 384-well format, with enough purified recombinant **GDP-loaded KRAS(G12C) Isoform A**, GTP, exchange factor SOS1 (son of sevenless homolog 1) (amino acids 564-1049), effector protein RBD-cRAF (amino acids 50-140), assay buffer and additives for 400 reactions.

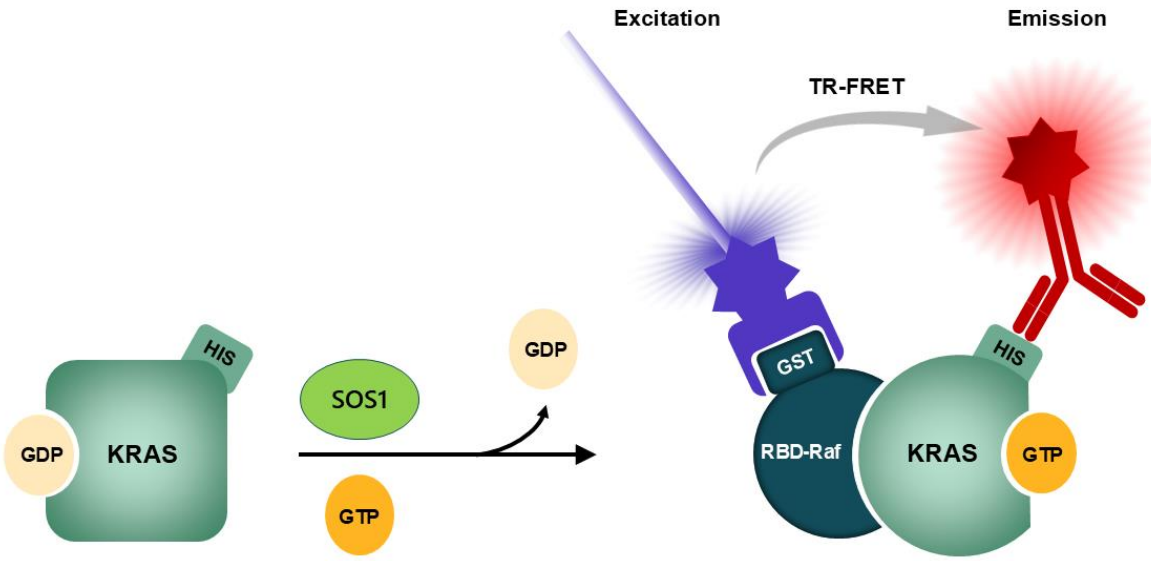

Figure 1: Illustration of the KRAS (G12C) Isoform A Coupled Nucleotide Exchange TR-FRET Assay Kit principle.
A sample containing GDP-loaded KRAS(G12C) Isoform A is incubated with SOS1 and GTP, allowing nucleotide exchange. GDP-loaded KRAS(G12C) Isoform A is in an inactive state and does not interact with the Ras-binding domain (RBD) of cRAF. SOS1 assists in the release of GDP from KRAS(G12C) so that GTP can occupy the nucleotide binding pocket. This results in a conformational change in KRAS(G12C) that permits its binding to RBD-cRAF. RBD-cRAF is then added to the reaction. After incubation, acceptor and donor dyes are added. The TR-FRET signal generated can be measured using a fluorescence plate reader. The signal intensity is proportional to the presence of GTP-bound KRAS(G12C).

Background

It is well established that RAS mutations are responsible for more than 30% of human cancers. KRAS(G12C) is one of the KRAS mutations that is found frequently in lung and colon carcinomas. The G12C mutation favors the activated (GTP-bound) state of KRAS, amplifying signaling pathways that lead to oncogenesis. Recent studies have led to the discovery of a small molecule called AMG510 (Amgen) that locks KRAS conformation in the GDP-bound (inactive) state, thereby blocking KRAS(G12C)-mediated signaling pathway. Compounds that affect the nucleotide exchange (GDP to GTP) reaction could lead to a novel approach leading to the inhibition of tumor cell growth in KRAS(G12C) driven tumors.

Applications

Screen small molecule inhibitors or antagonists that affect KRAS(G12C) nucleotide-binding status in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100640	KRAS (G12C), Isoform A, His-Tag, GDP-Loaded*	5 µg	-80°C
101573	SOS1, FLAG-Tag*	50 µg	-80°C
100519	RBD-cRAF, GST-tag*	5 µg	-80°C
79861-2	Guanosine 5'-triphosphate (GTP), (10 mM)	0.5 ml	-20°C
82710	RBD-RAS Binding Buffer (Incomplete)	2 x 3 ml	-20°C
	Tb-labeled donor	2 x 10 µl	-20°C
	Anti-6His Acceptor Dye	2 x 10 µl	-20°C
82735	0.5 M DTT	200 µl	-20°C
79311	3x Immuno Buffer 1	4 ml	-20°C
79969	White, nonbinding, low volume microtiter plate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
- Adjustable micropipettor and sterile filter tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed. Avoid multiple freeze/ thaw cycles!

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- This kit is compatible with up to 1% final DMSO concentration.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" wells.
- We recommend preincubating GDP-loaded KRAS(G12C) with inhibitors if the inhibition mechanism is similar to AMG-510; however, it is acceptable to add the GTP and SOS1 without the preincubation step.
- We recommend maintaining the diluted protein on ice during use.

- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using BI-2865 (#82711) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1x, 1x and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1:

1. Prepare **Complete RBD-RAS Binding Buffer** by adding 6 µl of 0.5M DTT to 3 ml of RBD-RAS Binding Buffer (Incomplete). Mix well.

2. Thaw **GDP-loaded KRAS(G12C)** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

3. Dilute **GDP-loaded KRAS(G12C)** to 3 ng/µl with **Complete RBD-RAS Binding Buffer** (4 µl/well).

4. Add 4 µl of diluted **GDP-loaded KRAS(G12C)** to each well.

5. Prepare the Test Inhibitor (2 µl per well): for a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 10 µl.

   5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in Complete RBD-RAS Binding Buffer, 5-fold more concentrated than the desired final concentrations.

   For the positive control and blank, use Complete RBD-RAS Binding Buffer (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 20-fold in Complete RBD-RAS Binding Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

   Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in Complete RBD-RAS Binding Buffer to keep the concentration of DMSO constant.

   For positive control and blank, prepare 5% DMSO in Complete RBD-RAS Binding Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

6. Add 2 µl of 5-fold intermediate serial dilutions of the Test Inhibitor to the “Test Inhibitor” wells.

7. Add 2 µl of Diluent Solution to the “Blank” and “Positive Control” wells.

8. Briefly centrifuge the plate and incubate for 30 minutes at Room Temperature (RT).

9. Thaw **GTP (10 mM)** and keep it on ice.

10. Thaw **SOS1** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

11. Dilute **SOS1** to a concentration of 120 ng/µl with Complete RBD-RAS Binding Buffer (1 µl/well).

12. Prepare a GTP and SOS1 Mix (2 µl/well): N wells × (1 µl of diluted SOS1 + 1 µl of 10 mM GTP).

13. Initiate the exchange reaction by adding 2 µl of GTP and SOS1 Mix to the "Test Inhibitor" and "Positive Control" wells.

14. Add 2 µl of Complete RBD-RAS Binding Buffer to the "Blank" wells.

15. Briefly centrifuge the plate and incubate at RT for 30 minutes.

16. Thaw **RBD-cRAF** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

17. Dilute **RBD-cRAF** to a concentration of 3.6 ng/µl with Complete RBD-RAS Binding Buffer (2 µl/well).

18. At the end of the 30 minutes incubation with SOS1/GTP, initiate the reaction by adding 2 µl of the diluted **RBD-cRAF** to all wells.

19. Briefly centrifuge the plate and incubate at RT for 30 minutes.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Diluted GDP-loaded KRAS(G12C) (3 ng/µl)	4 µl	4 µl	4 µl
Test Inhibitor	-	-	2 µl
Diluent Solution	2 µl	2 µl	-
30 minutes at Room Temperature			
GTP and SOS1 Mix	-	2 µl	2 µl
Complete RBD-RAS Binding Buffer	2 µl	-	-
30 minutes at Room Temperature			
Diluted RBD-cRAF (3.6 ng/µl)	2 µl	2 µl	2 µl
Total	**10 µl**	**10 µl**	**10 µl**

Step 2:

1. Dilute **3X Immuno Buffer** 3-fold with deionized water to prepare 1X Immuno Buffer.

2. Dilute **Tb-labeled Donor** and **Anti-6His Acceptor Dye** 200-fold, together, with 1x Immuno Buffer (you will need 10 µl of Donor/Acceptor Mix per well).

   Note: Make only enough as needed for the assay; store the remaining stock solution in aliquots at -20°C (minimum volume of 5 µl/ aliquot).

3. Add 10 µl of Donor/Acceptor Mixture to each well.

4. Incubate at RT for 60 minutes with slow agitation.

5. Read the TR-FRET signal in a microtiter-plate reader under settings described below (settings may need optimization depending on the instrument).

6. The "Blank" value should be subtracted from all other values.

Instrument Settings

Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

Reading Mode	Time Resolved
Excitation Wavelength	340 (20 nm bandwidth)
Emission Wavelength	620 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340 (20 nm bandwidth)
Emission Wavelength	665 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS

Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

$$FRET = \frac{S_{665}}{S_{620}}$$

When percentage activity is calculated, the FRET value from the Blank (it is expected that Blank and Negative Control have a similar values) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\%\ Activity = \frac{FRET_S - FRET_{blank}}{FRET_P - FRET_{blank}} \times 100\%$$

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{blank}$ = FRET value for the Blank, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example Results

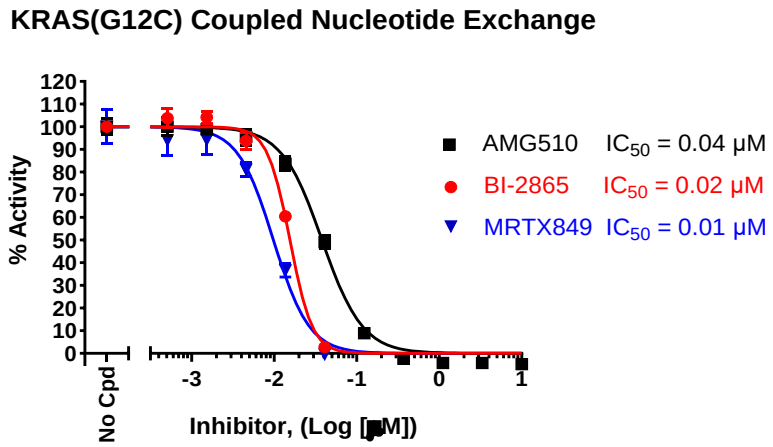

Figure 2: Effect of KRAS inhibitors on the nucleotide exchange of KRAS(G12C).
Inhibition of the nucleotide exchange of KRAS(G12C) was evaluated in the presence of increasing concentrations of BI-2865 (#82711), a pan-KRAS inhibitor, and the KRAS(G12C) specific inhibitors MRTX849 (SelleckChem #S8884) and AMG510 (#82712).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Canon J., *et al.,* 2019. *Nature* 575: 217-223.
Hillig R.C., *et al., 2019 PNAS USA* 116 (7): 2551-2560

Related Products

Products	*Catalog #*	*Size*
KRAS(G12V) Nucleotide Exchange Assay Kit	78519	384 reactions
KRAS(G12D) Nucleotide Exchange Assay Kit	78355	384 reactions
KRAS(G12D) Isoform A Coupled Nucleotide Exchange TR-FRET Assay Kit	82713	384 reactions
KRAS(G12C) Coupled Nucleotide Exchange Assay Kit	78004	384 reactions
KRAS (G12C), Isoform A, His-Tag, BODIPY-GDP Loaded	100537	20 µg
KRAS (G12C), Isoform A, His-Tag, GDP-Loaded	100640	4 x 50 µg

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