Description

The KRAS Isoform B Coupled Nucleotide Exchange Assay Kit is designed for screening and profiling of KRAS Isoform B antagonists/inhibitors by monitoring the binding of an effector protein such as the Ras binding domain of Raf1, (RBD-cRAF) to KRAS. The KRAS Isoform B Coupled Nucleotide Exchange Assay Kit comes in a convenient 384-well format, with enough purified recombinant **GDP-loaded KRAS Isoform B**, GTP, exchange factor SOS1 (sons of sevelenss homolog 1), an effector protein RBD-cRAF, assay buffer and additives for 400 reactions.

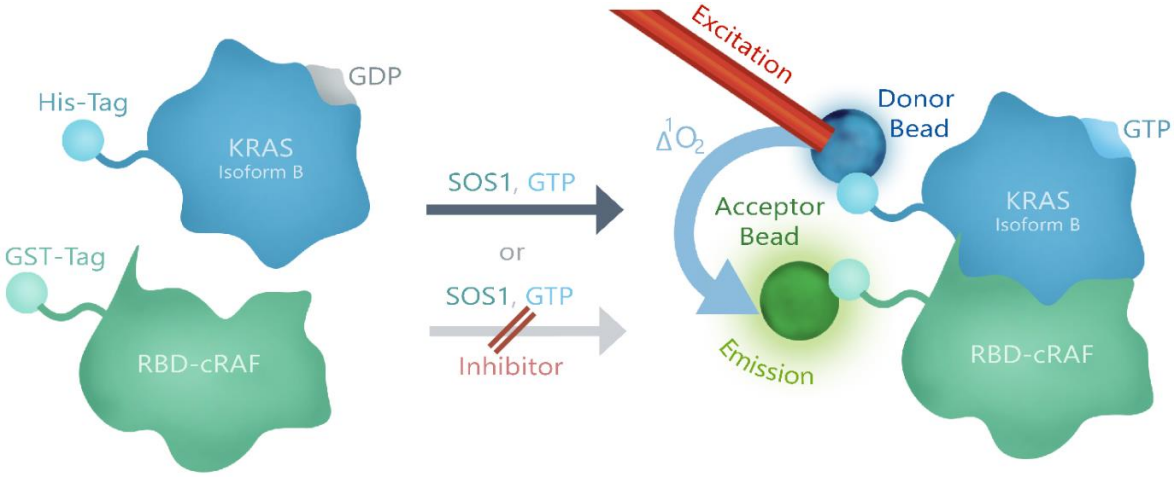

Figure 1: Illustration of the KRAS Isoform B Coupled Nucleotide Exchange Assay Kit principle.

SOS1 (son of sevenless) is a guanine nucleotide exchange factor that facilitates the exchange of GDP for GTP. GDP-loaded KRAS Isoform B 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 Isoform B so that GTP can occupy the nucleotide binding pocket. This results in a conformational change in KRAS Isoform B that permits its binding to RBD-cRAF. The KRAS Isoform B Coupled Nucleotide Exchange Assay Kit utilizes GST-tagged RBD-cRAF and His-tagged KRAS Isoform B to assay binding of KRAS to RBD-cRAF in the Alpha assay. Glutathione acceptor and Ni chelate donor beads are brought into proximal range by the binding of KRAS and RBD-cRAF, enabling the energy transfer from the donor to acceptor beads after laser excitation.

Background

High levels of wild-type KRAS cause a slowing of cell replication and growth, and increased apoptosis. This can be induced by cellular stress, certain types of radiation, chemical signals, and other prompts. Wild-type KRAS may also protect against mutant KRAS over-activation by dimerizing with mutant KRAS protein. The study of differences in the behavior of the wild-type and mutated forms of KRAS is especially important since KRAS mutations are responsible for more than 30% of human cancers. Compounds that affect the nucleotide exchange (GDP to GTP) reaction provide a novel approach to the inhibition of tumor cell growth in KRAS-driven tumors. In 2021, the Food and Drug Administration granted approval for use of AMG510 (Sotorasib**)**, a potent KRAS G12C inhibitor for non-squamous non-small cell lung cancer.

Application(s)

- Screen small molecule inhibitors or antagonists that affect KRAS Isoform B nucleotide-binding status in high throughput (HTS) applications.
- Counter-screen for the compounds that affect mutated forms of KRAS.

Supplied Materials

Catalog #	Name	Amount	Storage
101522	GDP-loaded KRAS Isoform B, His-Tag*	20 µg	-80°C
101573	SOS1, FLAG-Tag*	50 µg	-80°C
100519	RBD-cRAF, GST-Tag*	5 µg	-80°C
79861-2	GTP (10 mM)	0.5 ml	-20°C
82710	RBD-RAS Binding Buffer (Incomplete)	2 x 3 ml	-20°C
82735	0.5 M DTT	2 x 200 µl	-20°C
79311	3x Immuno Buffer 1	4 ml	-20°C

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

Materials Required but Not Supplied

Name	Ordering Information
AlphaLISA® Glutathione acceptor beads, 5 mg/ml	PerkinElmer #AL109C
AlphaScreen® Nickel Chelate donor beads, 5 mg/ml	PerkinElmer #AS101D
Optiplate - 384	PerkinElmer #6007290
AlphaScreen® microplate reader	
Adjustable micropipettor and sterile tips	

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.

Assay Principle

AlphaLISA® immunoassays are no-wash alternatives to ELISA immunoassays using a proprietary system developed by PerkinElmer. These homogeneous assays are robust, and they are ideal for a minimal hands-on approach. The Nickel-coated Alpha donor bead binds to the His-tagged KRAS Isoform B protein, while the glutathione-coated AlphaLISA® acceptor beadbinds to the GST-tag on RBD-cRAF. Glutathione acceptor and Ni chelate donor beads are brought into proximal range by the binding of KRAS Isoform B and RBD-cRAF, enabling the energy transfer from the donor to acceptor beads after laser excitation.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (λ=520-620 nm), such as Trypan Blue.
- Avoid the use of potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}).

- The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. Media like MEM, which lacks these components, does not affect AlphaScreen assays.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Negative Control", "Positive Control" and "Test Inhibitor" wells.
- We recommend preincubating the GDP-loaded KRAS Isoform B with inhibitors; however, it is acceptable to add the GTP and SOS1 without the preincubation step.
- We recommend using BAY-293 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.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

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

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

3. Dilute **GDP-loaded KRAS Isoform B** to 12 ng/µl in **Complete RBD-RAS Binding Buffer** (4 µl/ well).

4. Add 4 µl/well of diluted **GDP-loaded KRAS Isoform B** (12 ng/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 "Positive Control" and "Negative 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** in Complete **RBD-RAS Binding Buffer** to a concentration of 120 ng/µl (1 µl/ well).
12. Prepare a GTP and SOS1 Mix (2 µl/well): N wells × (1 µl of diluted SOS1 + 1 µl of GTP (10 mM)).
13. Initiate the exchange reaction by adding 2 µl of GTP/SOS1 Mix prepared as described above to the "Test Inhibitor" and the "Positive Control" wells.
14. Add 2 µl of Complete **RBD-RAS Binding Buffer** to the "Negative Control" 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** in **Complete RBD-RAS Binding Buffer** to a concentration of 3.6 ng/µl (2 µl/ well).
18. At the end of the 30-minute incubation with SOS1/GTP Mix, initiate the reaction by adding 2 µl of the diluted **RBD-cRAF** (3.6 ng/µl, 7.2 ng/well) to all wells. The final reaction volume is 10 µl.
19. Briefly centrifuge the plate and incubate at RT for 30 minutes.

Component	**Negative Control**	**Positive Control**	**Test Inhibitor**
Diluted GDP-loaded KRAS Isoform B (12 ng/µl)	4 µl	4 µl	4 µl
Test Inhibitor	-	-	2 µl
Diluent Solution	2 µl	2 µl	-
Centrifuge and incubate	30 minutes at Room Temperature		
GTP (10 mM)/SOS1 (120 ng/µl) Mix	-	2 µl	2 µl
Complete RBD-RAS Binding Buffer	2 µl	-	-
Centrifuge and incubate	30 minutes at Room Temperature		
Diluted RBD-cRAF (3.6 ng/µl)	2 µl	2 µl	2 µl
Centrifuge and incubate	30 minutes at Room Temperature		
Total	**10 µl**	**10 µl**	**10 µl**

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

21. Dilute the Glutathione Acceptor beads (PerkinElmer #AL109C) and the Nickel chelate Donor beads (PerkinElmer #AS101D) together at 1:500 and 1:250 respectively in 1x Immuno Buffer (i.e., for 400 reactions, ~8 ml of the detection reagent is needed = 16 µl of Glutathione Acceptor beads + 32 µl of Nickel Donor beads + 8 ml of 1x Immuno Buffer).

Protect your samples from direct exposure to light. Photobleaching will occur.

22. Add 20 µl of acceptor/donor beads mixture to all the wells.

23. Incubate 30 minutes at RT.

24. Read Alpha-counts using a compatible plate reader (PerkinElmer).

Example Results

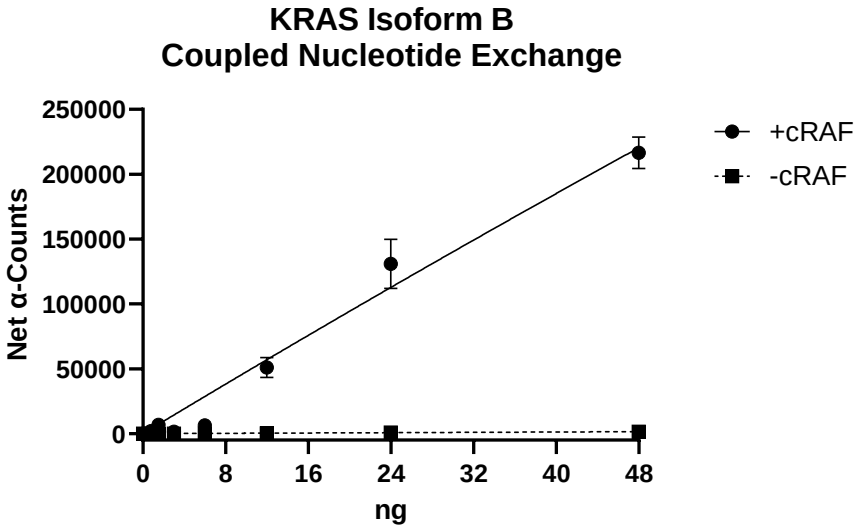

Figure 1: Nucleotide exchange of KRAS Isoform B.
The nucleotide exchange of KRAS Isoform B was evaluated in the presence or absence of cRAF.

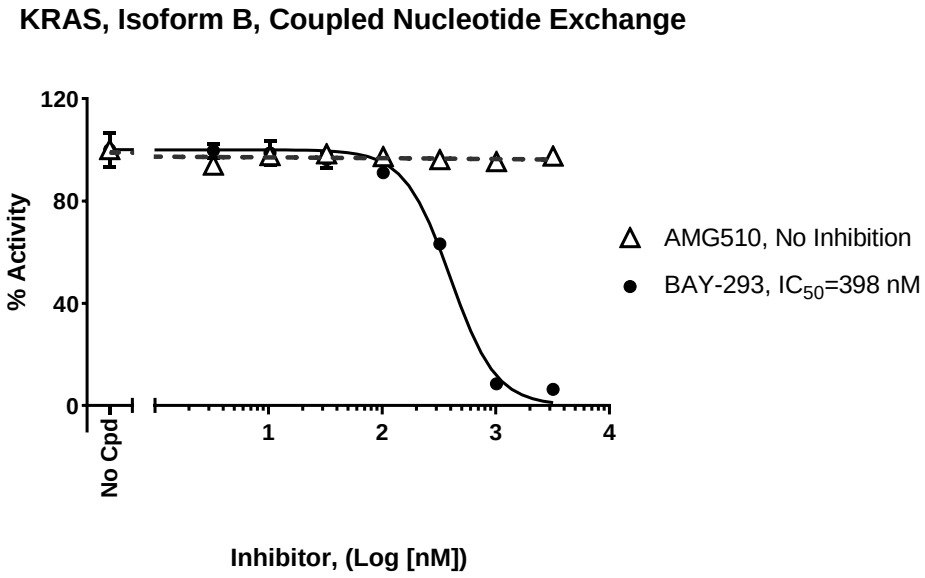

Figure 2: Effect of KRAS inhibitors on the nucleotide exchange of KRAS Isoform B.
Inhibition of the nucleotide exchange of KRAS Isoform B was evaluated in the presence of increasing concentrations of BAY-293 and AMG510 using KRAS Isoform B Coupled Nucleotide Exchange Assay Kit (BPS Bioscience #78583). BAY-293 selectively inhibits the KRAS-SOS1 interaction. AMG510 is a covalent inhibitor of KRAS G12C that does not affect wild-type KRAS.
Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General Considerations

Plates and Instruments: A plate reader capable of Alpha technology detection is required. We recommend using PerkinElmer 384-Optiplate #6007290 or EnSpire Alpha 2390 Mutilabel Reader.

The negative Control and Positive Control are important to determine the range of the assay. We recommend performing all assays in duplicate.

Troubleshooting Guide

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

References

Vasta J.D., *et al.,* 2022 *Nature Chem Biol* 18(6): 596-604.
Nuevo-Tapioles C, Philips M.R. 2022 *Front Cell Dev Biol* 10: 10333348.

Related Products

Products	*Catalog #*	*Size*
KRAS(G12C) Nucleotide Exchange Assay Kit	79859	384 reactions
KRAS(G12D) Nucleotide Exchange Assay Kit	78355	384 reactions
KRAS(G12V) Nucleotide Exchange Assay Kit	78519	384 reactions
KRAS(G12C) Coupled Nucleotide Exchange Assay Kit	78565	384 reactions

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