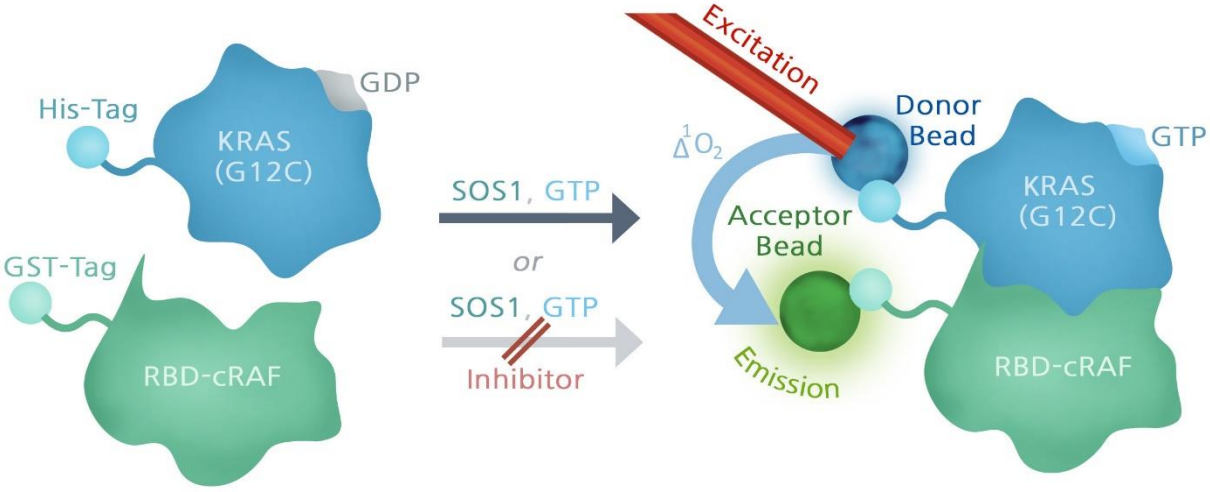
Description

The KRAS(G12C) Coupled Nucleotide Exchange Assay Kit is designed for screening and profiling of KRAS(G12C) antagonists/inhibitors by monitoring the binding of an effector protein (i.e. Ras binding domain of Raf1, RBD-cRaf) to KRAS(G12C). The KRAS(G12C) Coupled Nucleotide Exchange Assay Kit comes in a convenient 384-well format, with enough purified recombinant **GDP-loaded KRAS(G12C) Isoform A**, GTP, exchange factor SOS1, an effector protein RBD-cRAF, assay buffer and additives for 400 reactions. With this kit, a few simple steps on a microtiter plate are required for nucleotide exchange detection. First, a sample containing GDP-loaded KRAS(G12C) is incubated with SOS1 and GTP for the nucleotide exchange. Next, RBD-cRAF is added and incubated for the effector-RAS binding. Then, acceptor and donor beads are added and incubated for detection followed by reading the Alpha-counts.

SOS1 is a guanine nucleotide exchange factor that facilitates the exchange of GDP for GTP. GDP-loaded KRAS(G12C) is in an inactive state, and does not interact with the Ras-binding domain (RBD) of cRAF. SOS1 assists in the release GDP from KRAS(G12C) so that GTP can occupy the nucleotide binding pocket. This results in a conformational change in KRAS(G12C) that permits binding of RBD-cRAF to KRAS(G12C). The KRAS(G12C) Coupled Nucleotide Exchange Assay Kit utilizes GST-tagged RBD-cRAF and His-tagged KRAS (G12C) to assay binding of KRAS(G12C) to RBD-cRAF in the Alpha assay. Glutathione acceptor and Ni chelate donor beads are brought into proximal range by the binding of KRAS(G12C) and RBD-cRAF, enabling the energy transfer from the donor to acceptor beads after laser excitation.



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 cancers. 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.

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Applications

1. Screen small molecule inhibitors or antagonists that are expected to affect KRAS(G12C) nucleotide-binding status for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100640	GDP-loaded KRAS(G12C), Isoform A, His-tag*	10 µg	-80°C
100753	SOS1, FLAG-Tag, Avi-Tag, Biotin-Labeled*	2 x 100 µg	-80°C
100519	RBD-cRAF, GST-tag*	5 µg	-80°C
79861-2	GTP (10 mM)	0.5 ml	-20°C
78351	RBD-RAS Binding Buffer	6 ml	4°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	Catalog #
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 1 year from date of receipt when the materials are stored as directed.

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 the no-wash alternatives to ELISA immunoassays using the 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 is bound to a His-tagged KRAS(G12C) protein, while the glutathione-coated AlphaLISA® acceptor bead is binds to the GST-tag on a second protein, RBD-cRAF. With the presence of an analyte, the beads move close to one another and initiate excitation and emission that is then measured.

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Contraindications

Green and blue dyes, such as Trypan Blue, absorb light in the AlphaScreen® signal emission range (520-620 nm). Avoid the use of the 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. MEM, which lacks these components, does not affect AlphaScreen® assays.

The final concentration of DMSO in the reaction should not exceed 1%.

Assay Protocol

All samples and controls should be tested in duplicate. We recommend preincubating the GDP-loaded KRAS(G12C) with inhibitors if the inhibition mechanism is similar to AMG510; however, it is acceptable to add the GTP and SOS1 without the preincubation step.

The assay requires a Positive Control and a Negative Control in addition to the Test Inhibitor.

1. Thaw **GDP-loaded KRAS(G12C)** on ice. Briefly spin the tube containing the protein to recover the full content of the tube. Aliquot **GDP-loaded KRAS(G12C)** into single use aliquots. Store the remaining undiluted protein in aliquots at -80°C immediately. *Note: **GDP loaded KRAS(G12C)** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots and do not re-use the diluted protein.*

2. Dilute **GDP-loaded KRAS(G12C)** to 500 nM in **RBD-RAS binding buffer**. Note: the concentration of **GDP-loaded KRAS(G12C)** provided may vary. Verify the concentration of the **GDP-loaded KRAS(G12C)** written on the tube and dilute accordingly (*e.g*. If the stock concentration of **GDP-loaded KRAS(G12C)** is 10 µM, dilute 25 µl of **GDP-loaded KRAS(G12C)** in 475 µl **RBD-RAS binding buffer** for 400 reactions. *Prepare only the amount required for the assay. Discard any unused diluted KRAS(G12C).*

3. Prepare the Master Mix (6 µl/well): N wells × (1 µl of **diluted GDP-loaded KRAS(G12C), 500 nM** + 5 µl of **RBD-RAS binding buffer**). Add 6 µl of master mix to each well.

4. Prepare the Test Inhibitor (1 µl per well): for a titration, prepare serial dilutions at a concentration 10-fold higher than the desired final concentration. The final volume of the reaction is 10 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in deionized water, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use deionized water (Diluent Solution).

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 200-fold the highest desired concentration in DMSO (*e.g*. if the highest testing concentration is 50 µM, prepare a 10 mM solution in 100% DMSO). Then dilute the inhibitor 20-fold in deionized water to prepare the highest concentration of the 10X intermediate solution (i.e. to test at 50 µM, prepare a 500 µM intermediate solution by adding 5 µl of 10 mM inhibitor solution to 95 µl of deionized water). The concentration of DMSO is now 5%. Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 5% DMSO in deionized water to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 5% DMSO in water (vol/vol) as a 10X intermediate so that all

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wells contain the same amount of DMSO (Diluent Solution). *Caution: do not exceed 5% DMSO in the 10X intermediate solution.*

5. Add 1 µl of 10X intermediate serial dilutions of the Test Inhibitor to the testing wells. Add 1 µl of 5% DMSO (diluted in water) to the positive and negative control wells or add deionized water if the Test Inhibitor was dissolved in water.

6. Briefly centrifuge the plate and incubate for 30 minutes at room temperature.

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

8. Thaw **SOS1** on ice. Briefly spin the tube containing the protein to recover the full content of the tube. Aliquot SOS1 into single use aliquots. Store the aliquots at -80°C immediately. *Note: SOS1 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots and do not re-use the diluted protein.*

9. Dilute **SOS1** in **RBD-RAS binding buffer** to a concentration of 5 µM. Note: the concentration of SOS1 provided may vary. Verify the concentration of the protein written on the tube and dilute accordingly (*e.g*. If the stock concentration of **SOS1** is 20 µM, dilute 125 µl of **SOS1** in 375 µl of **RBD-RAS binding buffer** for 400 reactions). *Caution: prepare only the amount required for the assay. Discard any unused diluted SOS1.*

10. Combine **GTP** (10 mM) and diluted **SOS1** (5 µM) at a 1:1 ratio (2 µl/well) : N wells × (1 µl of diluted SOS1 (5µM) + 1 µl of GTP (10 mM)).

11. 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. For the "Negative control", add 2 µl of **RBD-RAS binding buffer** only.

12. Briefly centrifuge the plate and incubate at room temperature for 30 minutes.

13. Thaw **RBD-cRAF** on ice. Briefly spin the tube containing the protein to recover the full content of the tube. Aliquot **RBD-cRAF** into single use aliquots. Store aliquots at -80°C immediately. *Note: **RBD-cRAF** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots and do not re-use the diluted protein.*

14. Dilute **RBD-cRAF** in **RBD-RAS binding buffer** to a concentration of 25 nM. Note: the concentration of **RBD-cRAF** provided may vary. Verify the concentration of **RBD-cRAF** written on the tube and dilute it accordingly (*e.g*. If the stock concentration of **RBD-cRAF** is 25 µM, dilute 1 µl of **RBD-cRAF** in 999 µl **RBD-RAS binding buffer** for 400 reactions. Due to the relatively high concentration of the **RBD-cRAF**, an excess amount of the protein is supplied for your convenience). *Caution: prepare only the amount required for the assay. Discard unused diluted RBD-cRAF.*

15. At the end of the 30-minute incubation with SOS1/GTP, initiate the reaction by adding 1 µl of the diluted **RBD-cRAF** (25 nM) to all wells. The final reaction volume is 10 µl.

16. Briefly centrifuge the plate and incubate at room temperature for 30 minutes.

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Component	Negative Control	Positive Control	Test Inhibitor
Master Mix	6 µl	6 µl	6 µl
Test Inhibitor	-	-	1 µl
Diluent Solution	1 µl	1 µl	-
Centrifuge and incubate	30 minutes at room temperature		
GTP(10 mM)/SOS1(5 µM) mixture	-	2 µl	2 µl
RBD-RAS Binding Buffer	2 µl	-	-
Centrifuge and incubate	30 minutes at room temperature		
RBD-cRAF (25 nM)	1 µl	1 µl	1 µl
Total	**10 µl**	**10 µl**	**10 µl**

17. Dilute **3X Immuno Buffer** in deionized water to prepare 1X Immuno buffer by adding one volume of 3X Immuno Buffer to two volumes of deionized water.

18. Dilute the Glutathione Acceptor beads (PerkinElmer #AL109C) and the Nickel chelate Donor beads (PerkinElmer #AS101D) at 1:500 and 1:250 respectively in 1x Immuno buffer (e.g. for 400 reactions, ~8 mL of the detection reagent is needed. Therefore add 16 µl of Glutathione Acceptor beads and 32 µl of Nickel Donor beads to 8 mL of 1X Immuno buffer).

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

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

20. Incubate 30 min at room temperature.

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

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Example Results

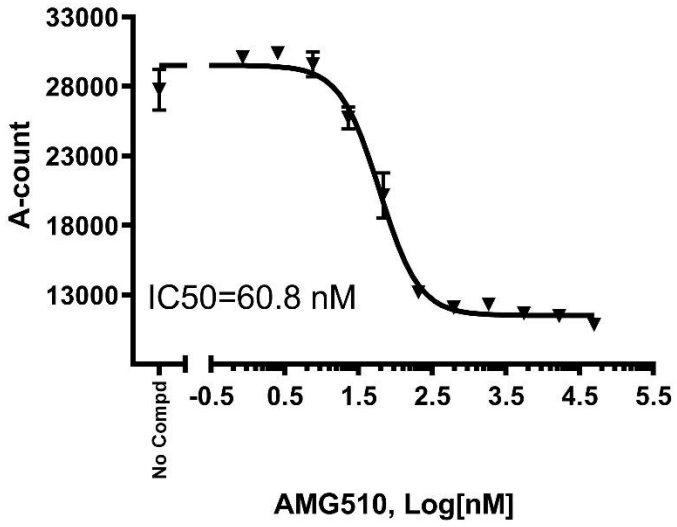

Figure 1: Effect of AMG510 on the nucleotide exchange of KRAS(G12C). Inhibition of the nucleotide exchange of KRAS(G12C) was evaluated in the presence of increasing concentrations of AMG510 using KRAS(G12C) Coupled Nucleotide Exchange Assay Kit (BPS Bioscience #78004).

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 doing these in duplicate.

Troubleshooting Guide

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

References

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

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Related Products

Products	*Catalog #*	*Size*
KRAS (G12C), Isoform A, His-Tag, GDP-Loaded	100640	4 x 50 µg
SOS1, FLAG-Tag, Avi-Tag, Biotin-Labeled	100753	various
KRAS(G12C) Nucleotide Exchange Assay Kit	79859	384 reactions
KRAS(G12D) Nucleotide Exchange Assay Kit	78355	384 reactions
KRAS (G12C), Isoform A, His-Tag (*E. coli*-derived)	100413	100 µg
KRAS (G12D), Isoform A, His-Tag	100623	100 µg
KRAS (G12V), Isoform B, His-Tag	100480	100 µg
KRAS (G13D), Isoform B, His-Tag	100479	100 µg
KRAS, Isoform B, His-Tag	11308	100 µg
p120GAP, His-Tag	100518	100 µg
KRAS (G12C), Isoform A, His-Tag, GppNHp-Loaded	100641	4x60 µg
KRAS (G12C), Isoform A, BODIPY-GDP Loaded, His-Tag	100537	4x60 µg

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