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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The KRAS(G12D) Coupled Nucleotide Exchange Assay Kit is designed for screening and profiling of KRAS(G12D) antagonists/inhibitors by monitoring the binding of an effector protein such as the Ras binding domain of Raf1, (RBD-cRaf) to KRAS(G12D). The KRAS(G12D) Coupled Nucleotide Exchange Assay Kit comes in a convenient 384-well format, with enough purified recombinant **GDP-loaded KRAS(G12D) Isoform A**, GTP, exchange factor SOS1, 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(G12D) 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 (son of sevenless) is a guanine nucleotide exchange factor that facilitates the exchange of GDP for GTP. GDP-loaded KRAS(G12D) 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(G12D) so that GTP can occupy the nucleotide binding pocket. This results in a conformational change in KRAS(G12D) that permits its binding to RBD-cRAF. The KRAS(G12D) Coupled Nucleotide Exchange Assay Kit utilizes GST-tagged RBD-cRAF and His-tagged KRAS (G12D) to assay binding of KRAS(G12D) to RBD-cRAF in the Alpha assay. Glutathione acceptor and Ni chelate donor beads are brought into proximal range by the binding of KRAS(G12D) and RBD-cRAF, enabling the energy transfer from the donor to acceptor beads after laser excitation.

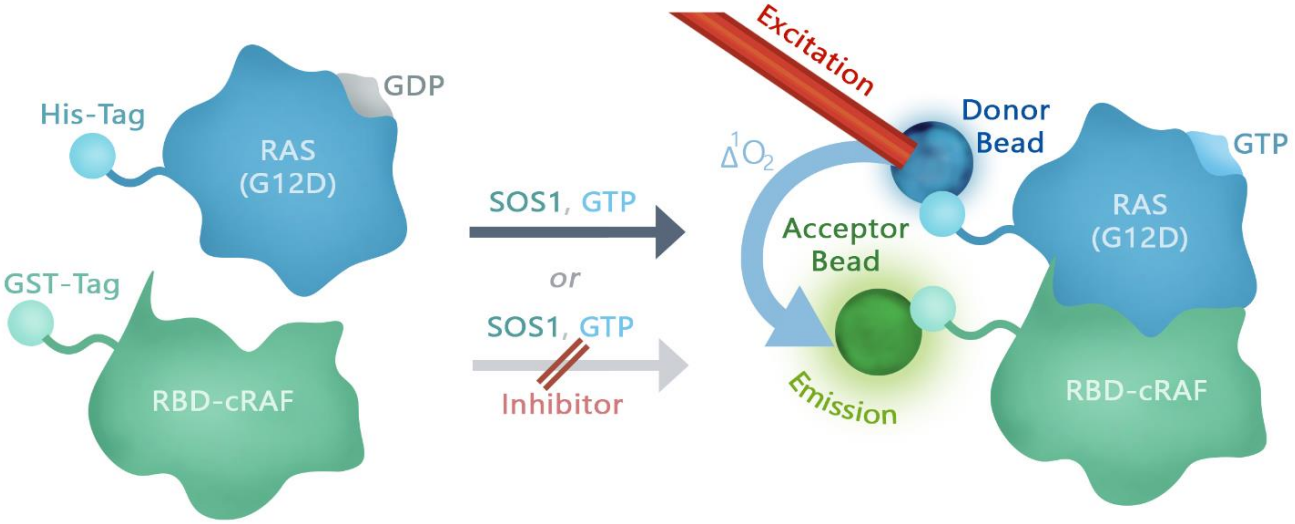

Figure 1: Illustration of the assay principle.

Background

It is well established that RAS mutations are responsible for more than 30% of human cancers. KRAS(G12D) is the most common mutation (33%) among KRAS mutant tumors. The G12D 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 MRTX1133 (Mirati) that locks KRAS conformation in the GDP-bound inactive state, thereby blocking KRAS(G12D)-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(G12D) driven tumors.

Applications

Screen small molecule inhibitors or antagonists that affect KRAS(G12D) nucleotide-binding status in high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
101312	GDP-loaded KRAS(G12D), Isoform A, His-Tag*	5 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
	RBD-RAS Binding Buffer (Incomplete)	2 x 3 ml	4°C
	DTT (0.5 M)	2 x 200 µl	
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 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 binds to the His-tagged KRAS(G12D) protein, while the glutathione-coated AlphaLISA® acceptor bead is binds to the GST-tag on RBD-cRAF. Glutathione acceptor and Ni chelate donor beads are brought into proximal range by the binding of KRAS(G12D) and RBD-cRAF, enabling the energy transfer from the donor to acceptor beads after laser excitation.

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(G12D) with inhibitors if the inhibition mechanism is similar to MRTX1133; 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. Prepare **Complete RBD-RAS Binding Buffer**: add 6 µl of 0.5M DTT to 3 ml of RBD-RAS Binding Buffer (Incomplete). Mix well.

2. Thaw **GDP-loaded KRAS(G12D)** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

3. Dilute **GDP-loaded KRAS(G12D)** to 3 ng/µl in **Complete RBD-RAS binding buffer**.

   *Note: The concentration of **GDP-loaded KRAS(G12D)** provided may vary. Verify the concentration of the **GDP-loaded KRAS(G12D)** written on the tube and dilute accordingly. Prepare only the amount required for the assay. Discard any unused diluted KRAS(G12D).*

4. Aliquot any unused (non-diluted) **GDP-loaded KRAS(G12D)** into single use aliquots. Store the remaining undiluted protein in aliquots at -80°C immediately.

   *Note: **GDP loaded KRAS(G12D)** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots and do not re-use the diluted protein.*

5. Add 4 µl/well of diluted **GDP-loaded KRAS(G12D)** (12 ng/well).

6. 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.

   Without DMSO

   a. 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 and negative controls, use **Complete RBD-RAS binding buffer** (Diluent Solution).

   Or

   With DMSO

   a. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration that is 100-fold higher than the highest desired concentration in DMSO (*i.e.*, if the highest testing concentration is 50 µM, prepare a 5 mM solution 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 solution (i.e., to test at 50 µM, prepare a 250 µM intermediate solution by adding 5 µl of 5 mM inhibitor solution to 95 µl of **Complete RBD-RAS binding buffer**). The concentration of DMSO is now 5%.

   b. Prepare serial dilutions of the Test Inhibitor at concentrations 5-fold higher than the desired final concentrations using 5% DMSO in **Complete RBD-RAS binding buffer** to keep the concentration of DMSO constant.

   c. For positive and negative controls, prepare 5% DMSO in **Complete RBD-RAS binding buffer** (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Caution: Do not exceed 5% DMSO in the 5-fold intermediate solution.

7. Add 2 µl of 5-fold intermediate serial dilutions of the Test Inhibitor to the testing wells.

8. Add 2 µl of 5% DMSO in Complete RBD-RAS binding buffer to the positive and negative control wells or add Complete RBD-RAS binding buffer if the Test Inhibitor was dissolved in buffer.

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

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

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

12. Dilute **SOS1** in Complete **RBD-RAS binding buffer** to a concentration of 120 ng/µl. *Note: The concentration of SOS1 provided may vary. Verify the concentration of the protein written on the tube and dilute accordingly.*

    Caution: Prepare only the amount required for the assay. Discard any unused diluted SOS1.

13. Aliquot unused (undiluted) 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.

14. Combine **GTP** (10 mM) and diluted **SOS1** (120 ng/ml) at a 1:1 ratio (2 µl/well): N wells × (1 µl of diluted SOS1 (120 ng/µl) + 1 µl of GTP (10 mM)).

15. 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 Complete **RBD-RAS binding buffer** only.

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

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

18. Dilute **RBD-cRAF** in Complete **RBD-RAS binding buffer** to a concentration of 1.8 ng/µl. *Note: The concentration of **RBD-cRAF** provided may vary. Verify the concentration of **RBD-cRAF** written on the tube and dilute it accordingly. 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.

19. Aliquot the remaining unused, undiluted **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.*

20. At the end of the 30-minute incubation with SOS1/GTP, initiate the reaction by adding 2 µl of the diluted **RBD-cRAF** (1.8 ng/µl or 3.6 ng/well) to all wells. The final reaction volume is 10 µl.

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

Component	Negative Control	Positive Control	Test Inhibitor
GDP-loaded KRAS(G12D) (3 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) mixture	-	2 µl	2 µl
Complete RBD-RAS Binding Buffer	2 µl	-	-
Centrifuge and incubate	30 minutes at room temperature		
RBD-cRAF (1.8 ng/µl)	2 µl	2 µl	2 µl
Centrifuge and incubate	30 minutes at room temperature		
Total	**10 µl**	**10 µl**	**10 µl**

22. 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.

23. 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 (i.e., 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.

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

25. Incubate 30 min at room temperature.

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

Example Results

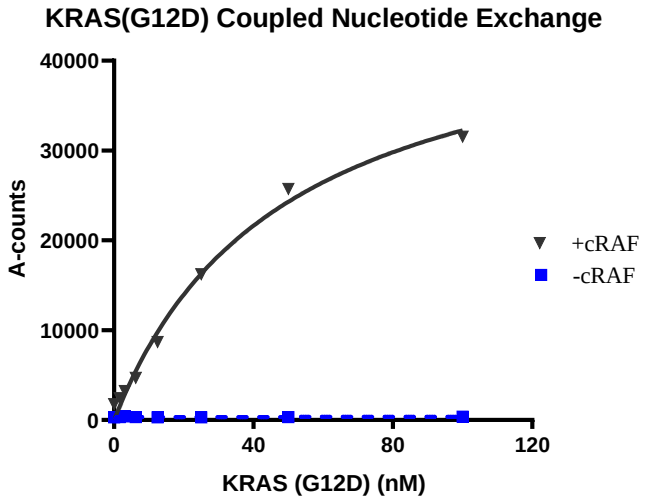

Figure 1: Nucleotide exchange of KRAS (G12D).
The nucleotide exchange of KRAS(G12D) was evaluated in the presence or absence of cRAF.

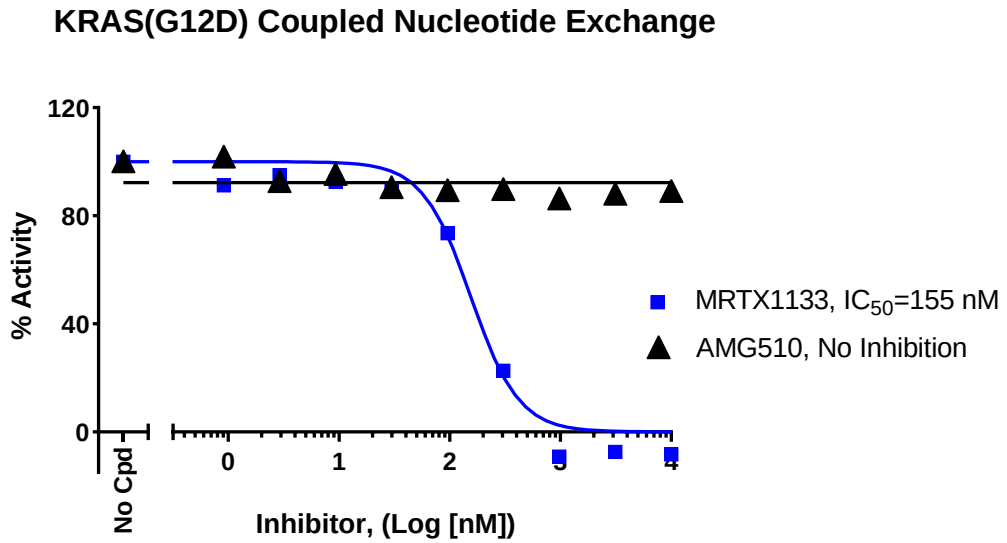

Figure 2: Effect of MRTX1133 and AMG510 on the nucleotide exchange of KRAS(G12D).
Inhibition of the nucleotide exchange of KRAS(G12D) was evaluated in the presence of increasing concentrations of KRAS(G12D) and KRAS (G12C)-specific inhibitors using KRAS(G12D) Coupled Nucleotide Exchange Assay Kit.
Data 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

Wang, X., *et al.* 2022. *J Med Chem* 65: 3123-33.
Hillig, R.C., *et al.* 2019. *PNAS USA* 116 (7): 2551-2560

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