SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The KRAS(G12V) Isoform B Coupled Nucleotide Exchange Assay Kit is designed for screening and profiling of antagonists/inhibitors of KRAS(G12V), Isoform B, by monitoring the binding of an effector protein such as the Ras binding domain of Raf1 (RBD-cRaf) to KRAS. The 384-well AlphaLISA® Assay Kit contains a sufficient amount of purified recombinant **GDP-loaded KRAS(G12V) Isoform B**, GTP, exchange factor SOS1, effector protein RBD-cRAF, assay buffer, and additives for 400 reactions. First, a sample containing GDP-loaded KRAS(G12V) Isoform B 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(G12V) 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(G12V) Isoform B so that GTP can occupy the nucleotide binding pocket. This results in a conformational change in KRAS that permits its binding to RBD-cRAF. The KRAS (G12V) Isoform B Coupled Nucleotide Exchange Assay Kit utilizes GST-tagged RBD-cRAF and His-tagged KRAS(G12V) Isoform B to assay the binding of KRAS to RBD-cRAF in the AlphaLISA® assay. Glutathione acceptor and Nickel 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.

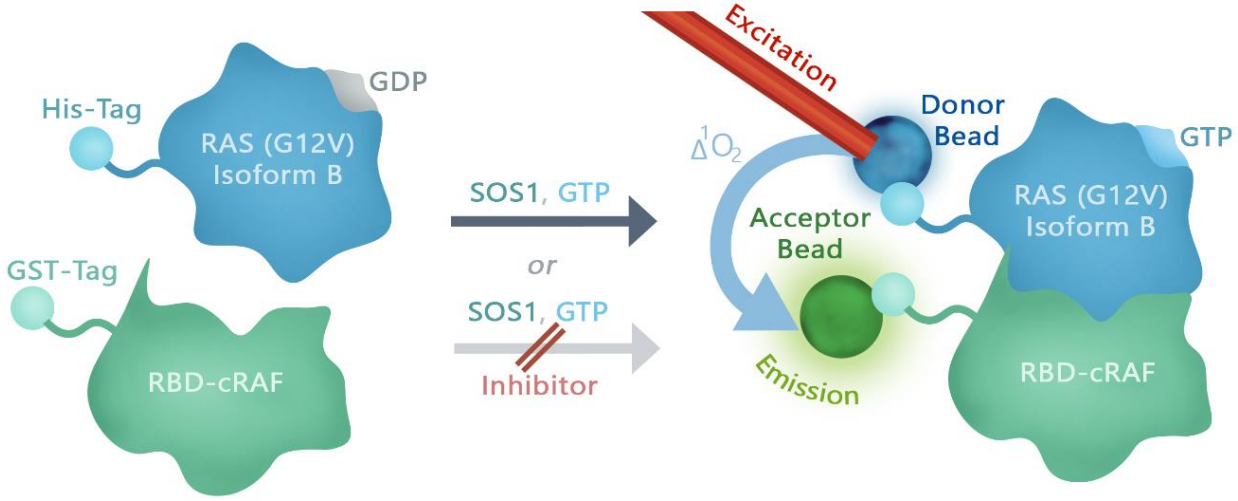

Figure 1: Illustration of the assay principle.

Background

High levels of wild-type KRAS cause a slowing of cell replication and growth, and an increased pace of apoptosis. This can be induced by cellular stress, certain types of radiation, chemical signals, and other prompts. The resulting KRAS reaction is thus considered a defensive mechanism to counteract the effects of over-activation of KRAS. 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 mutations are responsible for more than 30% of human cancers. 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 driven tumors.

Applications

- Screen small molecule inhibitors or antagonists that affect KRAS(G12V) Isoform B nucleotide-binding status in high throughput (HTS) applications.
- Counter-screen for compounds that affect mutated forms of KRAS.

Supplied Materials

Catalog #	Name	Amount	Storage
101355	GDP-loaded KRAS(G12V) 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
	RBD-RAS Binding Buffer (Incomplete)	2 x 3 ml	4°C
	DTT (0.5 M)	2 x 200 µl	-20°C
79311	3x Immuno Buffer 1	4 ml	-20°C

**The concentration of the proteins 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(G12V) Isoform B protein, while the glutathione-coated AlphaLISA® acceptor bead 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(G12V) Isoform B 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(G12V) Isoform B 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. 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(G12V) Isoform B** on ice. Briefly spin the tube containing the protein to recover the full content of the tube.

3. Dilute **GDP-loaded KRAS(G12V) Isoform B** to 12 ng/µl in **Complete RBD-RAS Binding Buffer**.

   *Note: The concentration of **GDP-loaded KRAS(G12V) Isoform B** provided may vary. Verify the concentration written on the tube and dilute accordingly. Prepare only the amount required for the assay. Discard any unused diluted KRAS(G12V) Isoform B.*

4. Aliquot any unused (non-diluted) **GDP-loaded KRAS(G12V) Isoform B** into single use aliquots. Store the remaining undiluted protein in aliquots at -80°C immediately.

   *Note: **GDP loaded KRAS(G12V) Isoform B** 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(G12V) Isoform B** (48 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 of the compound 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 100-fold 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 5-fold 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 Diluent Solution (for example 5% DMSO in complete RBD-RAS binding buffer) to the positive and negative control wells.

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/µl) 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 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 3.6 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** (3.6 ng/µl or 7.2 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(G12V) 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) mixture	-	2 µl	2 µl
Complete RBD-RAS Binding Buffer	2 µl	-	-
Centrifuge and incubate	30 minutes at room temperature		
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**

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

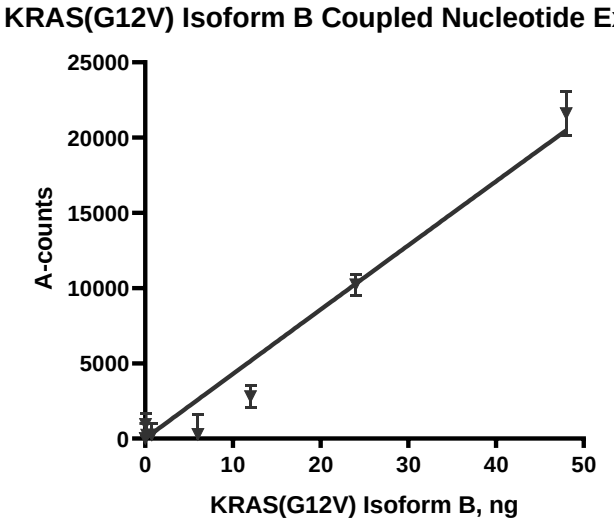

Figure 1: Nucleotide exchange of KRAS(G12V) Isoform B.
The nucleotide exchange of KRAS(G12V) Isoform B was evaluated in the presence of cRAF.

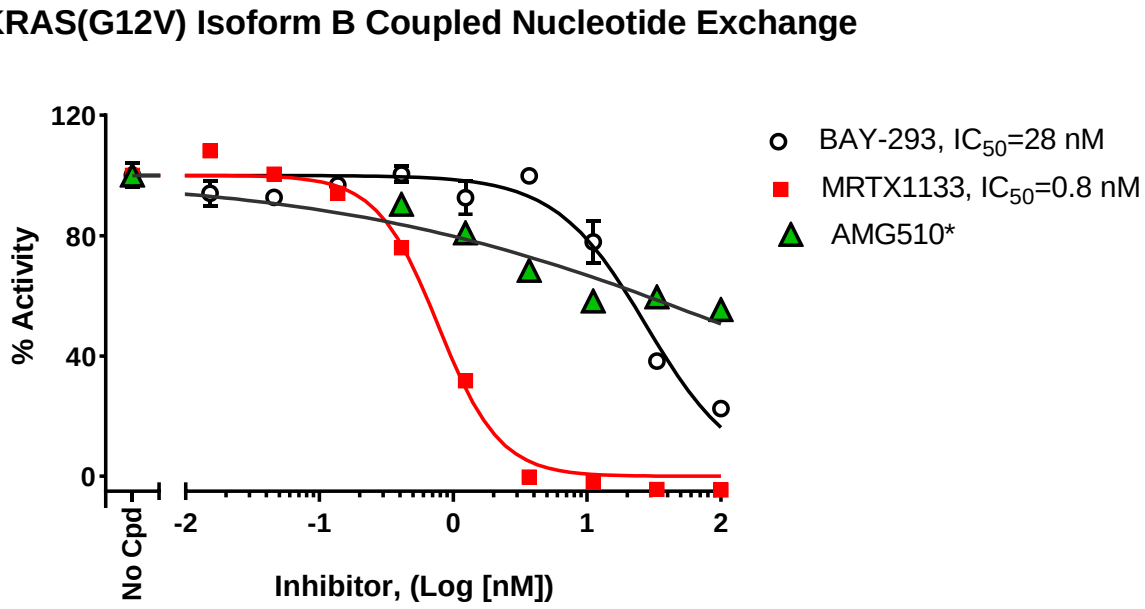

Figure 2: Effect of KRAS inhibitors on the nucleotide exchange of KRAS(G12V) Isoform B.
Inhibition of the nucleotide exchange of KRAS(G12V) Isoform B was evaluated in the presence of increasing concentrations of BAY-293, MRTX1133 and AMG510 using KRAS(G12V) Isoform B Coupled Nucleotide Exchange Assay Kit (BPS Bioscience #78825). BAY-293 selectively inhibits the KRAS-SOS1 interaction. MRTX1133 selectively inhibits KRAS(**G12D**) mutant. AMG510 is a covalent inhibitor of KRAS(**G12C**). *K_{inact}/K_i not determined.

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

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	4 x 60 µg
KRAS (G12C), Isoform A, BODIPY-GDP Loaded, His-Tag	100537	4 x 60 µg