MANUAL

AlphaLISA® KRAS G12C / SOS1 Binding Kit

Product number: AL3151 C/F

Research Use Only. Not for use in diagnostic procedures.

Product Information

Application: This kit is designed to assess inhibitors of KRAS G12C and SOS1 binding using a homogeneous no wash AlphaLISA assay.

Sensitivity: $IC_{50:}$ 461 nM (average, using BI-2852).

Signal to background ratio: 189 using 20 nM KRAS G12C protein, 1 nM of SOS1 protein and 10µM GTP

Kit contents: The kit contains 6 components: anti-6xHis AlphaLISA Acceptor beads, Glutathione Alpha Donor beads, GTP, KRAS G12C protein, SOS1 protein and AlphaLISA PPI buffer.

Storage: The kit components must be stored at 4 ˚C in the dark. Reconstituted reagents can be aliquoted (not under 10µL) then frozen stored at – 80 °C for 1 month. Avoid multiple freeze-thaw cycles.

Stability: This kit is stable for at least 12 months from the manufacturing date when stored in its original packaging and the recommended storage conditions.

Analyte of Interest

KRAS is a small GTPase implicated in various biological processes, such as cell proliferation, cell survival, and cell metabolism. This proto-oncogene is well known to be mutated in many cancer subtypes, inducing an uncontrolled proliferation and cell metabolism modifications. It thereby contributes to the Warburg effect in cancer cells. Like the majority of small GTPases, KRAS binds to GDP in its inactive form or binds to GTP to switch into active form. KRAS G12C is one of the most commonly present mutant forms in cancer which leads to a permanently active state of KRAS.

The Ras guanine nucleotide exchange factor, also called SOS1, is a GEF protein promoting the active form of KRAS. The upregulation of the KRAS/SOS1 interaction leads to cancer phenotypes.

Identifying new KRAS/SOS1 inhibitors or GTP competitors are therefore the two major strategies to control biological processes involved in cancer growth, by reducing the KRAS activity as well as the associated pathways.

Description of the AlphaLISA Assay

The AlphaLISA detection of KRAS G12C to SOS1 binding uses anti-6xHis AlphaLISA® acceptor beads to capture the His tagged SOS1 protein and Glutathione donor beads to capture the GST-tagged KRAS G12C protein. Donor beads and acceptor beads come into proximity in presence of GTP. Excitation of the Donor beads leads to the release of singlet oxygen that triggers a cascade of energy transfer reactions in the Acceptor beads, resulting in a sharp peak of light emission at 615 nm (Figure 1).

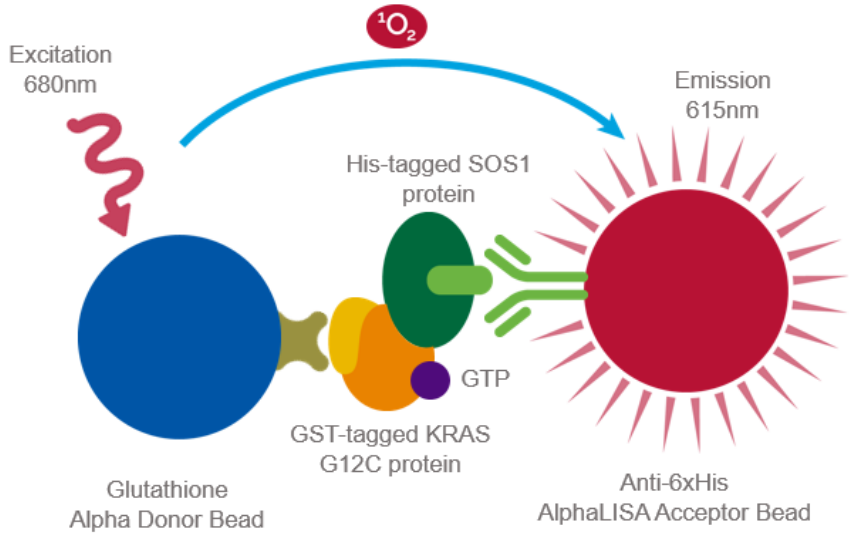


Figure 1. AlphaLISA Assay Principle.

Precautions

- The Alpha Donor beads are light-sensitive. All the other assay reagents can be used under normal light conditions. All Alpha assays using the Donor beads should be performed under subdued laboratory lighting (< 100 lux). Green filters (LEE 090 filters (preferred) or Roscolux filters #389 from Rosco) can be applied to light fixtures.
- All blood components and biological materials should be handled as potentially hazardous.

Kit Content: Reagents and Materials

Kit components	AL3151C*** (500 assay points)	AL3151F*** (5000 assay points)
AlphaLISA 6his Acceptor beads stored in PBS, 0.05% Kathon, pH 7.2	40 µL @ 5 mg/mL (1 brown tube, white cap)	400 µL @ 5 mg/mL (1 brown tube, white cap)
AlphaLISA Glutathione Donor beads stored in PBS, 0.05% Kathon, pH 7.2	40 µL @ 5 mg/mL (1 brown tube, black cap)	400 µL @ 5 mg/mL (1 brown tube, black cap)
GTP	53.32µg lyophilized (1 tube, clear cap)	53.32µg lyophilized (10 tubes, clear cap)
SOS1 protein (6his tagged)	0.59µg lyophilized (1 tube, clear cap)	0.59µg lyophilized (10 tubes, clear cap
KRAS G12C protein (GST tagged)	9.27µg lyophilized (1 tube, clear cap)	9.27µg lyophilized (10 tubes, clear cap)
AlphaLISA PPI Buffer (5X)	10 mL, 1 small bottle	100 mL, 1 large bottle

* Reconstitute GTP, SOS1 protein and KRAS G12C protein in 100 µL Milli-Q® grade H_2O respectively. The reconstituted reagents should be used within 60 minutes. After reconstitution, aliquot and store unused protein at -80 °C for 1 month. Avoid multiple freeze-thaw cycles.

** Extra buffer can be ordered separately (cat # AL015C: 10 mL, cat # AL015F: 100 mL).

*** The number of assay points is based on an assay volume of 20 µL in 384 well plates using the kit components at the recommended concentrations.

Sodium azide should **not** be added to the stock reagents. High concentrations of sodium azide (> 0.001 % final in the assay) might decrease the AlphaLISA signal.

Specific additional required reagents and materials:

The following materials are recommended:

Item	Suggested source	Catalog #
TopSeal™-A Adhesive Sealing Film	Revvity Inc.	6050185
AlphaPlate-384, Shallow Well (ProxiPlate)	Revvity Inc.	6008350 6008359
EnVision®-Alpha Reader	Revvity Inc.	-

The following reagents might be required for particular applications:

Item	Supplier	Catalog number
BI-2852	MedChemExpress	HY-126247 (10mM x 1mL in DMSO)

Recommendations

- The volume indicated on each tube is guaranteed for single pipetting. Multiple pipetting of the reagents may reduce the theoretical amount left in the tube. To minimize loss when pipetting beads, it is preferable not to pre-wet the tip.
- Centrifuge all tubes (including lyophilized analyte) before use to improve recovery of content (2000g, 10-15 sec). Re-suspend the beads by vortexing before use. Do not vortex the proteins.
- Use Milli-Q® grade H_2O to dilute 5X AlphaLISA PPI Buffer and to reconstitute the lyophilized reagents.
- When reagents are added to the microplate, make sure the liquids are at the bottom of the well.
- Small volumes may be prone to evaporation. It is recommended to cover microplates with TopSeal™-A Plus Adhesive Sealing Films to reduce evaporation during incubation. Microplates can be read with the TopSeal-A Film.
- The AlphaLISA signal is detected with an EnVision Multilabel Reader equipped with the Alpha option using the AlphaScreen standard settings (e.g. Total Measurement Time: 550 ms, Laser 680 nm Excitation Time: 180 ms, Mirror: D640as, Emission Filter: M570w, Center Wavelength 570 nm, Bandwidth 100 nm, Transmittance 75%).
- AlphaLISA signal will vary with temperature and incubation time. For consistent results, identical incubation times and temperature should be used for each plate.

Competition Assay Procedure

IMPORTANT: PLEASE READ THE RECOMMENDATIONS BELOW BEFORE USE

- The manual described below is an ***example*** for generating 250 assay points in a 20 µL final assay volume per point. These calculations do not include excess reagent to account for losses during transfer of solutions or dead volumes. If a different number of samples are tested, the volumes of all reagents must be adjusted accordingly.

- The dilution manual is provided for information only. As needed, the number of replicates or the range of concentrations covered can be modified.

One Incubation Step Manual described as below:

1. Preparation of 1X AlphaLISA PPI Buffer (for 10 mL):

   Add 2 mL of 5X AlphaLISA PPI Buffer to 8 mL H_2O.

2. Preparation of serial dilutions of BI-2852 (as example of tested compound) in 1X AlphaLISA PPI buffer as follows:

   a. Thaw the 10mM BI-2852 stock solution (ref HY-126247 from MedChemExpress)

b. Add 20µL of 10mM stock solution (100% DMSO) to 180µL of 1X AlphaLISA PPI buffer to obtain a 10X-concentrated BI-2852 solution with 10% DMSO. Prepare serial dilutions of 10X BI-2852 in 1x **AlphaLISA PPI buffer supplemented with 10% DMSO.** DMSO must stay at a constant concentration to avoid compound precipitation and assay interference.

Tube	Volume of standard	Volume of 1X buffer + or - 10% DMSO	[BI-2852] (µM) (10X)	[BI-2852] (µM) (1X)
A	20µL of 10mM stock	180 µL (no DMSO)	**1000**	**100**
B	60 µL of tube A	140 µL (10% DMSO)	**300**	**30**
C	60 µL of tube B	120 µL (10% DMSO)	**100**	**10**
D	60 µL of tube C	140 µL (10% DMSO)	**30**	**3**
E	60 µL of tube D	120 µL (10% DMSO)	**10**	**1**
F	60 µL of tube E	140 µL(10% DMSO)	**3**	**0.3**
G	60 µL of tube F	120 µL (10% DMSO)	**1**	**0.1**
H	60 µL of tube G	140 µL (10% DMSO)	**0.3**	**0.03**
I	60 µL of tube H	120 µL (10% DMSO)	**0.1**	**0.01**
J	60 µL of tube I	140 µL (10% DMSO)	**0.03**	**0.003**
K	60 µL of tube J	120 µL (10% DMSO)	**0.001**	**0.0001**
L	0	140 µL (10% DMSO)	**0**	**0**

3. Preparation of 5X 6His tagged SOS1 protein:
   a. Reconstitute lyophilized SOS1 protein in 100 µL H_2O to make a 100 nM SOS1 stock solution.
   b. Add 50 µL of the 100 nM SOS1 stock solution to 950 µL of 1X AlphaLISA PPI buffer to obtain a 5nM working solution of 6his-SOS1 protein.

   Prepare just before use.

NB: Serial dilutions and 6His tagged SOS1 protein must be dispensed in the microplate at this step and incubated for 1h at 23°C

4. Preparation of the 10X GST tagged KRAS G12C protein:
   a. Reconstitute lyophilized KRAS G12C protein in 100 µL H_2O to make a 2000 nM KRAS G12C stock solution.
   b. Add 50 µL of the 2000nM KRAS G12C stock solution to 450 µL of 1X AlphaLISA PPI buffer to obtain a 200nM working solution of GST-KRAS G12C protein.

   Prepare just before use.

5. Preparation of the 10X GTP solution:

   a. Reconstitute lyophilized GTP in 100 µL H_2O to make a 1mM stock solution.
   b. Add 50 µL of 1mM GTP protein to 450 µL 1X AlphaLISA PPI buffer to obtain a 100µM stock solution of GTP protein.

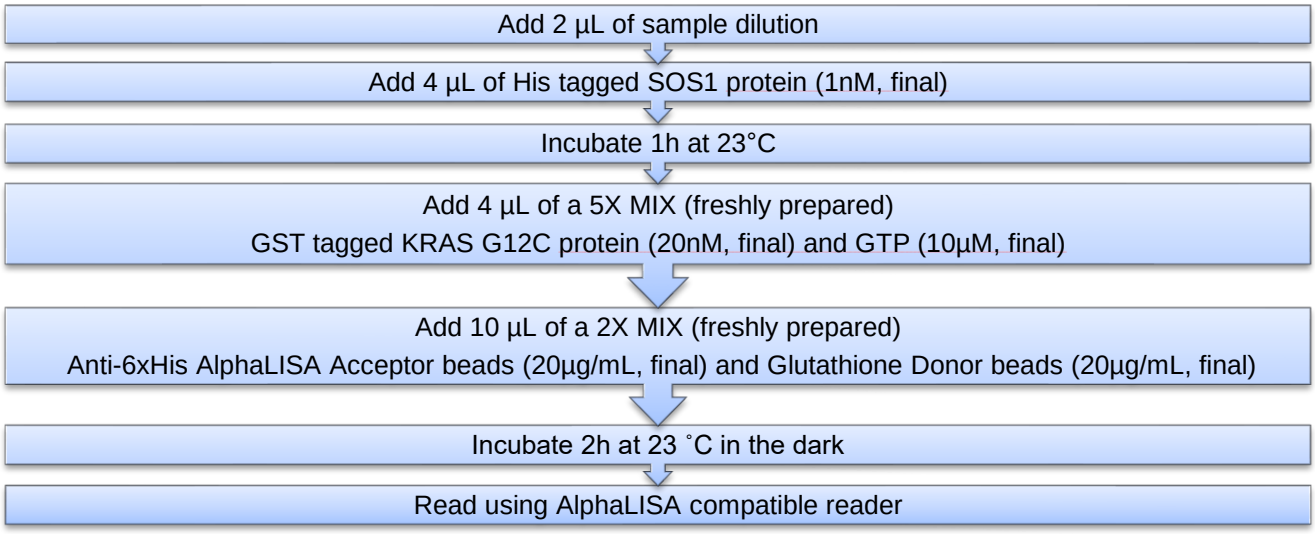
   Prepare just before use.

6. Preparation of the mixed GTP/GST-tagged KRAS G12C solution

    a. Add 500µl of GTP 10X and 500µl of GST tagged KRAS G12C protein 10X.
    Prepare just before use.

7. Preparation of the mixed 6his-Acceptor beads (40 µg/mL)/ Glutathione Donor beads (40 µg/mL) to obtain a mix of 2X Acceptor/Donor beads:
    a. Keep the beads under subdued laboratory lighting.
    b. Add 20 µL of 5 mg/mL 6his Acceptor beads and 20 □L of 5 mg/mL glutathione Donor beads to 2460 µL of 1X AlphaLISA PPI buffer
    Prepare just before use.

8. Distribute the prepared reagents in a shallow well AlphaPlate (384 wells):



Read Settings: AlphaLISA signal is detected using an EnVision Multilabel Reader equipped with the Alpha option using the following settings: Total Measurement Time: 550 ms, Laser: 680 nm, Excitation Time: 180 ms, Mirror: 640as (Barcode# 444), Emission Filter: Wavelength 570nm, bandwidth: 100nm, Transmittance 75%, (Barcode# 244).

Typical competitive binding Data:

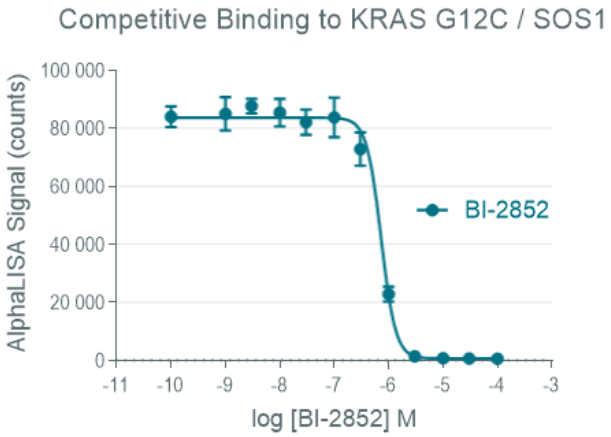


Figure 2. Competitive Binding: BI-2852 competitively bind to KRAS G12C / SOS1 with IC_{50} =647nM. IC_{50} value was calculated by using nonlinear regression fitting with GraphPad Prism 9.

Troubleshooting Guide

You will find below recommendations for common situations that you might encounter with your AlphaLISA binding assay. If further assistance is needed, do not hesitate to contact our technical support team for assistance.

Issue	Recommendations and Comments
High background signal	• Buffer is not freshly made. Make new. • Incubation time is longer than recommended range.
Low AlphaLISA signal	• Optimize EnVision with Plate format.
High variation between replicates or low Z' values	• Make sure that reagents are at the bottom of the well by tapping or swirling the plate gently on a smooth surface after each addition.

You will find detailed recommendations for common situations you might encounter with your AlphaLISA Assay kit at: www.revvity.com

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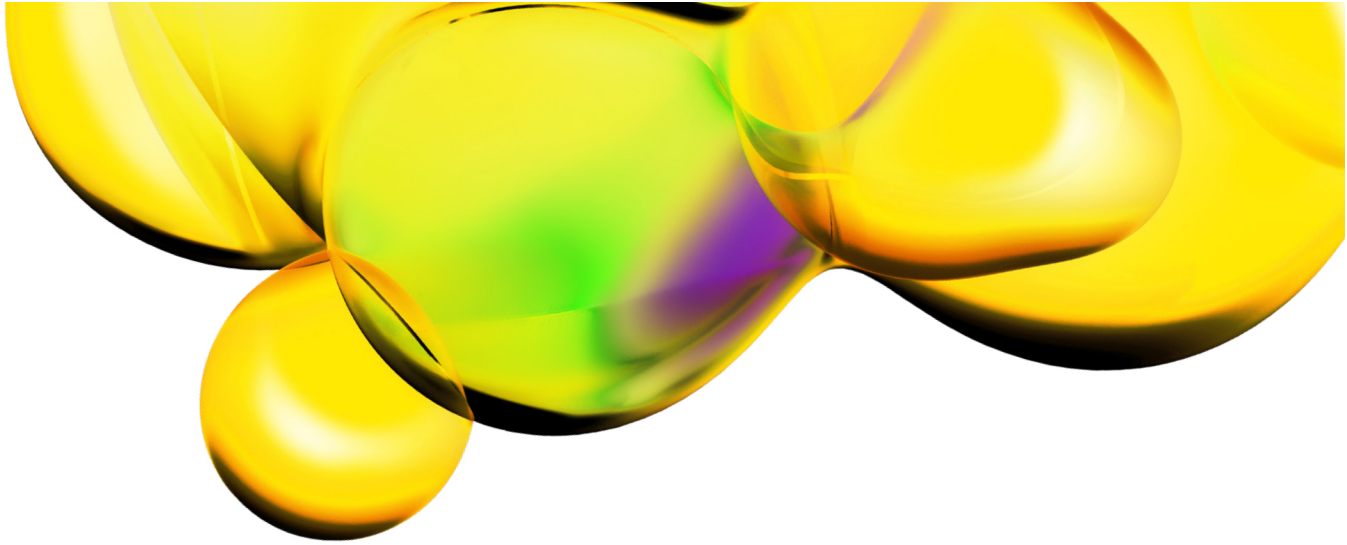

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