



KRAS-WT/SOS1 BINDING ASSAY KITS

PROTOCOL

Part # 63ADK000CB15PEG & 63ADK000CB15PEH

Test size: 500 tests (63ADK000CB15PEG), 10,000 tests (63ADK000CB15PEH) - assay volume: 20 μ L

Revision: 01 (February 2020)

Store at: -60°C or below

This product is intended for research purposes only. The product is not intended to be used for therapeutic or diagnostic purposes.

ASSAY PRINCIPLE

The HTRF KRAS-WT/SOS1 Binding Assay is designed to measure the interaction between KRAS-WT and SOS1 proteins. Utilizing HTRF (Homogeneous Time-resolved Fluorescence) technology, the assay enables simple and rapid characterization of compound and antibody blockers in a high throughput format.

As shown in Figure 1, the interaction between Tag1-SOS1 and Tag2-KRAS-WT is detected by using anti-Tag1-Terbium (HTRF donor) and anti-Tag2-XL665 (HTRF acceptor). When the donor and acceptor antibodies are brought into close proximity due to SOS1 and KRAS-WT binding, excitation of the donor antibody triggers fluorescent resonance energy transfer (FRET) towards the acceptor antibody, which in turn emits specifically at 665 nm. This specific signal is directly proportional to the extent of KRAS-WT/SOS1 interaction. Thus, compound or antibody blocking KRAS-WT/SOS1 interaction will cause a reduction in HTRF signal.

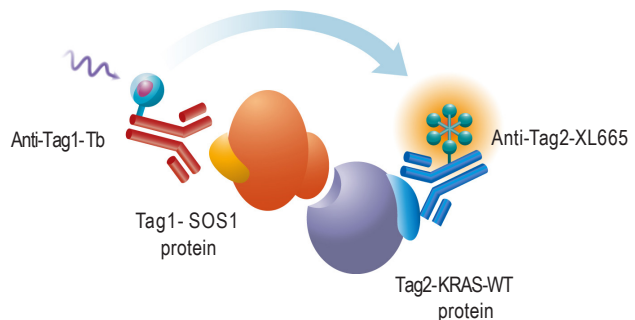
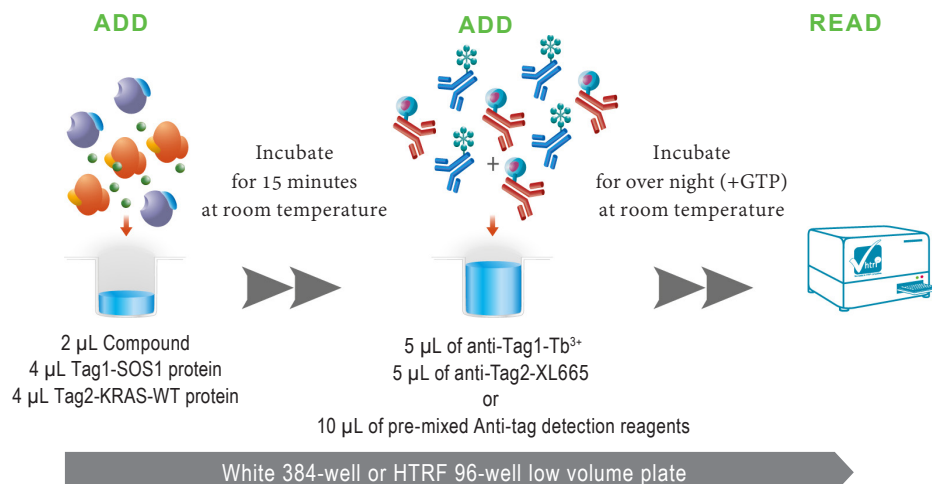


Figure 1: Principle of the HTRF KRAS-WT/SOS1 assay.

PROTOCOL AT A GLANCE



Make sure to use the setup for Tb³⁺ Cryptate. For more information about set-up and compatible HTRF® readers, please visit our website at: <http://www.cisbio.com/readers>

MATERIALS:

KIT COMPONENTS	500 TESTS CAT # 63ADK000CB15PEG	10,000 TESTS CAT # 63ADK000CB15PEH
Tag1-SOS1* MW: 58.8 kDa	1 vial 25 µL 500 X Frozen	1 vial 420 µL 500 X Frozen
Tag2-KRAS-WT* MW: 20.2 kDa	1 vial 25 µL 500 X Frozen	1 vial 420 µL 500 X Frozen
Anti-Tag1-Tb ³⁺	1 vial 25 µL 100 X Frozen	1 vial 500 µL 100 X Frozen
Anti-Tag2-XL665	1 vial 50 µL 50 X Frozen	1 vial Lyophilized
Diluent	1 vial 20 mL Cat# 62DLBDDF (200 mL) ready-to-use	1 vial 200 mL Cat# 62DLBDDF ready-to-use
Detection Buffer	1 vials 10 mL Cat# 62DB2FDG (130 mL) ready-to-use	1 vial 130 mL Cat# 62DB2FDG (130 mL) ready-to-use

* The amounts of Tag1-SOS1 and Tag2-KRAS-WT provided are sufficient for the validated amounts of tagged proteins suitable for compound inhibition study: optimized concentrations of SOS1 and KRAS-WT in 20 µL final assay volume.

Additional material (Not provided): GTP (SIGMA, V900868).

For reading, an HTRF®-Certified Reader is needed.

For HTRF microplate recommendations, please visit <http://www.cisbio.com/microplate-recommendations>

For a list of HTRF-compatible readers and setup recommendations, please visit <http://www.cisbio.com/readers>

STORAGE AND STABILITY

Store the kit at -60°C or below. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.



Reagents

Once thawed, tagged SOS1 & KRAS-WT stock solution may be frozen, and can be thawed only once. Once thawed (or reconstituted), anti-Tag solutions can be frozen once.

To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.

Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

REAGENT PREPARATION**BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature.
- Before use, allow all reagents to warm up to room temperature then homogenize buffer and diluent. It is recommended to filter buffers before use.
- The tagged protein solutions must be prepared in individual vials - DO NOT premix tagged solutions prior to dispensing.
- The anti-Tag solutions must be prepared in individual vials and can be premix prior to dispensing.
- Compounds may be prepared in diluent. We recommend keeping DMSO below 0.5% during the assay (20 µL final volume).






TO PREPARE WORKING SOLUTIONS:

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

500 TESTS	10,000 TESTS
Tag1-SOS1 protein 500 X stock solution of optimized final concentration	
Thaw the Tag1-SOS1 protein* solution. Dilute 100-fold the 500 X Tag1-SOS1 protein stock solution with diluent buffer to prepare a 5 X working solution. e.g. 5 µL of thawed Tag1-SOS1 protein stock solution + 495 µL of diluent buffer. (20 µL final volume).	
Tag2-KRAS-WT protein 500 X stock solution of optimized final concentration	
Thaw the Tag2-KRAS-WT protein* solution. Dilute 100-fold the 500 X Tag2-KRAS-WT protein stock solution with diluent buffer to prepare a 5 X working solution. e.g. 5 µL of thawed Tag2-KRAS-WT protein stock solution + 495 µL of diluent buffer. (20 µL final volume).	
Anti-Tag1-Tb³⁺	
Thaw the anti-Tag1-Tb ³⁺ solution. This 100 X stock solution can be frozen and stored at -60°C or below. Dilute 100-fold the 100 X anti-Tag1-Tb ³⁺ stock solution with detection buffer to prepare a 1 X working solution.	
e.g. 25 µL of thawed anti-Tag1-Tb ³⁺ stock solution + 2475 µL of detection buffer.	e.g. 0.5 mL of thawed anti-Tag1-Tb ³⁺ stock solution + 49.5 mL of detection buffer.
Anti-Tag2-XL665	
Thaw the anti-Tag2-XL665 solution. This 50 X stock solution can be frozen and stored at -60°C or below. Dilute 50-fold the 50 X anti-Tag2-XL665 stock solution with detection buffer. e.g. 50 µL of thawed anti-Tag2-XL665 stock solution + 2450 µL of detection buffer.	Reconstitute lyophilized anti-Tag2-XL665 with 1 mL of distilled water. This allows to a 50 X stock solution, that can be frozen and stored at -60°C or below. Dilute 50-fold the 50 X anti-Tag2-XL665 stock solution with detection buffer. e.g. 1.0 mL of reconstituted anti-Tag2-XL665 stock solution + 49.0 mL of detection buffer.

*Titration of Tag1-SOS1 or Tag2-KRAS-WT can be performed if necessary.

ASSAY PROTOCOL

Step 1		Dispense 2 µL of compound/antibody or diluent 4 µL of Tag1-SOS1 protein 4 µL of Tag2-KRAS-WT protein
Step 2		Incubate for 15 minutes at room temperature.
Step 3		Dispense 10 µL of pre-mixed anti-Tag1-Tb ³⁺ and anti-Tag2-XL665.
Step 4		Seal the plate and incubate for over night (+GTP) at room temperature.
Step 5		Remove the plate sealer and read on an HTRF® compatible reader.

STANDARD PROTOCOL FOR INHIBITORY ASSAY IN 20 μ L FINAL VOLUME

	Inhibitor	Tag1-SOS1	Tag2-KRAS-WT	Anti-Tag1-Tb ⁺	Anti-Tag2-XL665	Diluent	Detection buffer
Sample	2 μ L	4 μ L	4 μ L	5 μ L	5 μ L		
Positive control		4 μ L	4 μ L	5 μ L	5 μ L	2 μ L	
Negative control		4 μ L		5 μ L	5 μ L	6 μ L	
Cryptate control				5 μ L		10 μ L	5 μ L
Buffer control						10 μ L	10 μ L

EXAMPLE OF PLATE MAP

	1	2	3	4	5	6
A	Buffer control: 10 μ L diluent 10 μ L detection buffer	Repeat Well A1	Repeat Well A1	Compound...: 2 μ L compound... 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well A4	Repeat Well A4
B	Cryptate control: 10 μ L diluent 5 μ L detection buffer 5 μ L anti-Tag1-Tb	Repeat Well B1	Repeat Well B1	Compound...: 2 μ L compound... 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well B4	Repeat Well B4
C	Negative control: 6 μ L diluent 4 μ L Tag1-SOS1 10 μ L pre-mix anti-Tag reagents	Repeat Well C1	Repeat Well C1	Compound...: 2 μ L compound... 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well C4	Repeat Well C4
D	Positive control: 2 μ L diluent 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well D1	Repeat Well D1	Compound...: 2 μ L compound... 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well D4	Repeat Well D4
E	Compound 1: 2 μ L compound 1 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well E1	Repeat Well E1	Compound...:	Repeat Well E4	Repeat Well E4
F	Compound 2: 2 μ L compound 2 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well F1	Repeat Well F1	Compound...:	Repeat Well F4	Repeat Well F4
G	Compound...: 2 μ L compound... 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well G1	Repeat Well G1	Compound...:	Repeat Well G4	Repeat Well G4
H	Compound...: 2 μ L compound... 4 μ L Tag1-SOS1 4 μ L Tag2-KRAS-WT 10 μ L pre-mix anti-Tag reagents	Repeat Well H1	Repeat Well H1			

DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

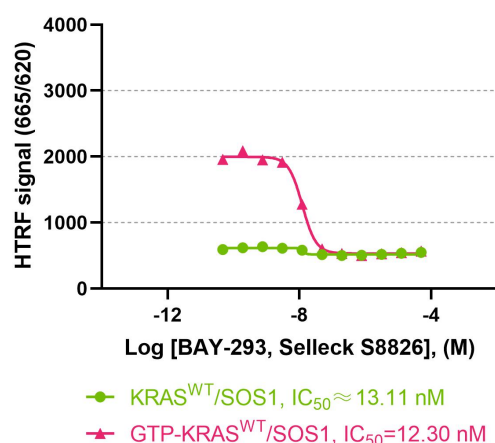
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit <http://www.cisbio.com/data-reduction>

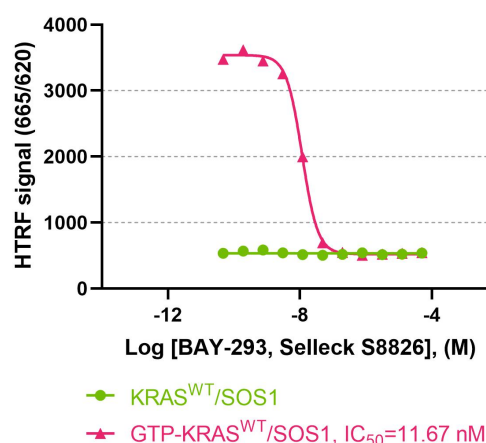
APPLICATION NOTE

To investigate the difference between nude KRAS and GTP bound KRAS, the IC_{50} values of inhibitors are measured in KRAS/SOS1 and GTP-KRAS/SOS1 binding assays.

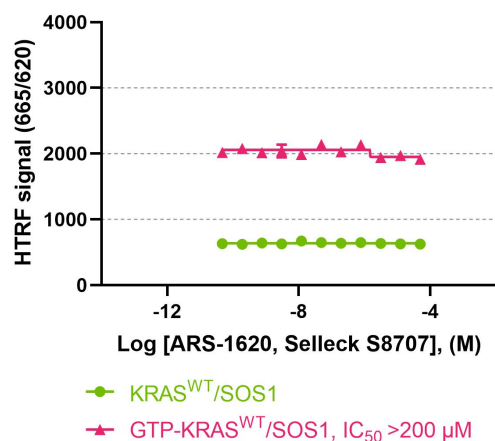
KRAS^{WT}/SOS1 binding assay
Inhibitory effect of SOS1 inhibitor
(Incubation for 2 h)



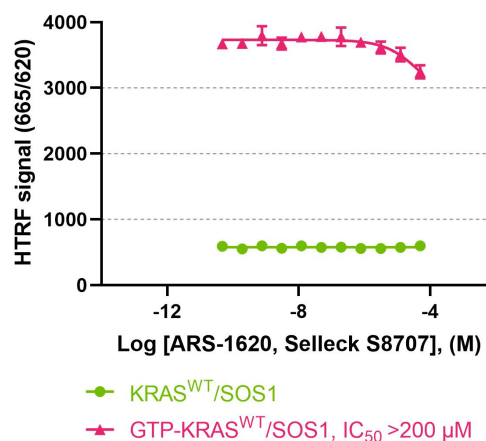
KRAS^{WT}/SOS1 binding assay
Inhibitory effect of SOS1 inhibitor
(Incubation for over night)



KRAS^{WT}/SOS1 binding assay
Inhibitory effect of KRAS(G12C) inhibitor
(Incubation for 2 h)



KRAS^{WT}/SOS1 binding assay
Inhibitory effect of KRAS(G12C) inhibitor
(Incubation for over night)



STANDARD PROTOCOL FOR GTP BOUND KRAS ASSAY IN 20 μ L FINAL VOLUME

To carry out GTP bound KRAS assay, please replace the working solution preparation step of Tag2-KRAS-WT protein with following steps:

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

Prepare 10 mM stock solution of GTP in distilled water. All guanosine phosphate derivatives are unstable and solutions should be made fresh immediately before use. Recommended final concentration of GTP: 10 μ M.

GTP bound Tag2-KRAS-WT protein 500 X stock solution of optimized final concentration

Thaw the Tag2-KRAS-WT protein* solution.
Dilute 100-fold the 500 X Tag2-KRAS-WT protein stock solution and 200-fold the 10 mM GTP stock solution with diluent buffer to prepare a 5 X working solution.
e.g. 5 μ L of Tag2-KRAS-WT protein stock solution + 2.5 μ L of 10 mM GTP stock solution + 492.5 μ L of diluent buffer. (20 μ L final volume).

Reagent	Final conc. (nM)	Working conc. (nM)	Stock conc. (nM)	Dilution ratio	Volume of Stock (μ L)	Volume of Diluent (μ L)	Volume of TOTAL (μ L)
Tag2-KRAS-WT protein	1 X	5 X	500 X	100	5	492.5	500.0
GTP	10,000	50,000	10,000,000	200	2.5		

RESULTS

The data shown below must not be substituted for the data obtained in the laboratory, and should be considered only as an example.

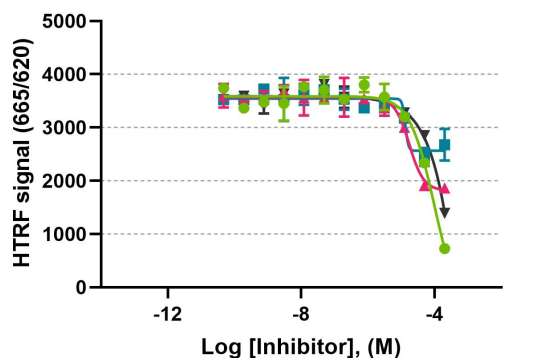
The inhibitory effects of small molecules were tested at optimized concentrations of SOS1 and KRAS-WT.

Readouts on PHERAstar FS with laser.

Note that results may vary from one HTRF[®] compatible reader to another.

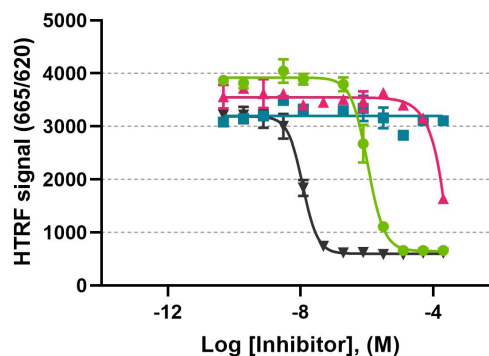
Inhibitor	Vender	Cat#	Description
AMG 510	Selleck	S8830	An effective covalent inhibitor of KRAS(G12C) which has potential anti-tumor activity.
6H05	Selleck	S7330	A selective, allosteric inhibitor of KRAS(G12C).
KRAS(G12C) inhibitor 12	Selleck	S7331	An allosteric inhibitor of KRAS(G12C).
ARS-1620	Selleck	S8707	An effective covalent inhibitor of KRAS(G12C) with oral activity.
KRpep-2d	Selleck	S8499	A selective inhibitory cyclic peptide of KRAS(G12D). Comparing with KRAS(WT) and KRAS(G12C), it has higher selectivity to KRAS(G12D).
AZ 628	Selleck	S2746	A novel inhibitor of pan-RAF.
PLX-4720	Selleck	S1152	An effective, selective inhibitor of BRAF(V600E).
BAY-293	Selleck	S8826	An effective covalent inhibitor of SOS1. It can selectively inhibit the KRAS/SOS1 interaction.

GTP-KRAS^{WT}/SOS1 binding assay
Inhibitory effect of small molecules
(Incubation for over night)



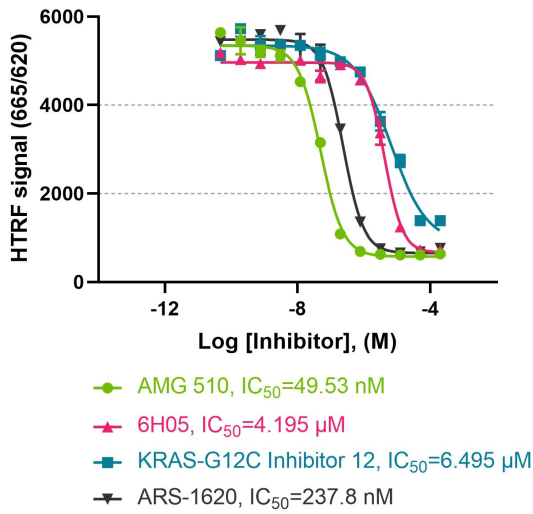
- AMG 510, IC_{50} =98.17 μ M
- ▲ 6H05, IC_{50} > 200 μ M
- KRAS-G12C Inhibitor 12, IC_{50} > 200 μ M
- ▼ ARS-1620, IC_{50} > 200 μ M

GTP-KRAS^{WT}/SOS1 binding assay
Inhibitory effect of small molecules
(Incubation for over night)

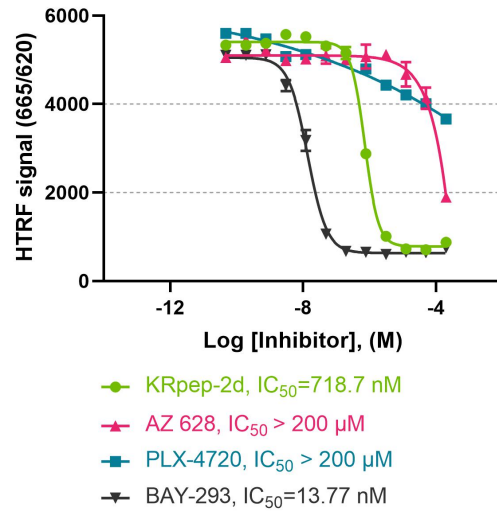


- KRpep-2d, IC_{50} =1.053 μ M
- ▲ AZ 628, IC_{50} > 200 μ M
- PLX-4720, IC_{50} > 200 μ M
- ▼ BAY-293, IC_{50} =11.65 nM

GTP-KRAS^{G12C}/SOS1 binding assay
Inhibitory effect of small molecules
(Incubation for over night)



GTP-KRAS^{G12C}/SOS1 binding assay
Inhibitory effect of small molecules
(Incubation for over night)



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