MANUAL

HTRF KRAS G12C/SOS1 PPI KITS

Part # 64KRASG12PEG & 64KRASG12PEH

Test Size#: 500 TESTS (64KRASG12PEG), 10,000 TESTS (64KRASG12PEH)

Revision: #03 of September 2023 **Store at:** ≤-60°C

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

ASSAY PRINCIPLE

The HTRF KRAS G12C/SOS1 PPI kit is designed to measure the interaction between KRAS G12C and SOS1. Utilizing HTRF (Homogeneous Time-resolved Fluorescence) technology, the assay enables simple and rapid characterization of GTP competitors and KRAS G12C /SOS1 inhibitors in a high throughput format.

As shown in Figure 1, the interaction between KRAS G12C and SOS1 is detected by using anti-Tag1 labelled with XL665 (HTRF acceptor) and anti-Tag2 labelled with Terbium cryptate (HTRF donor). When the donor and acceptor antibodies are brought into close proximity due to the KRAS G12C and SOS1 interaction, excitation of the donor antibody triggers fluorescence 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 / SOS1 interaction. Thus, GTP competitors and KRAS G12C /SOS1 inhibitors will cause a reduction in HTRF signal.

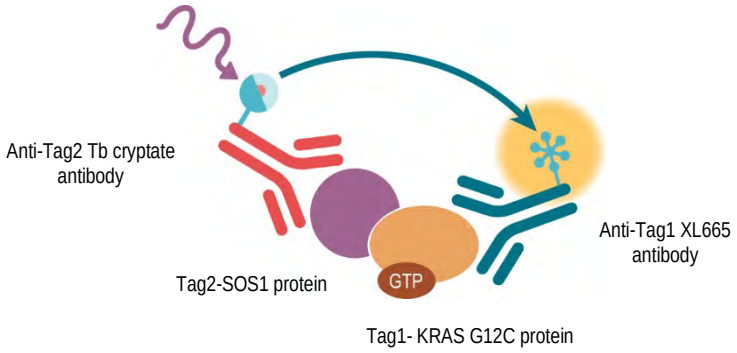
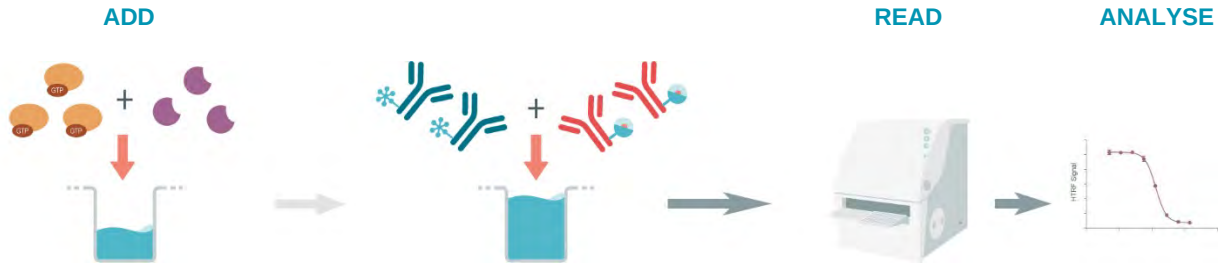


Figure 1: Principle of the HTRF KRAS G12C /SOS1 PPI kit.

MANUAL AT A GLANCE



Small volume white assay microplate

MATERIALS

KIT COMPONENTS	500 TESTS CAT # 64KRASG12PEG	10,000 TESTS CAT # 64KRASG12PEH
Tag1-Human KRAS G12C protein Frozen	1 vial - 50 µL 20X	1 vial - 1 mL 20X
Tag2- Human SOS1 Frozen	1 vial - 50 µL 40X	1 vial - 1 mL 40X
KRAS/SOS1 standard Frozen	1 vial - 30 µL 50mM	2 vials - 30 µL 50mM
Anti-Tag1 XL665 antibody Frozen	1 vial - 125 µL 20X	2 vials – 1.25 mL 20X
Anti-Tag2 Tb cryptate antibody Frozen	1 vial - 50 µL 50X	1 vial - 1 mL 50X
KRAS/SOS1 GTP Frozen	1 vial - 25 µL 40X	1 vial - 500 µL 40X
Binding domain detection buffer #2 Frozen	1 vial - 20 mL	2 vials - 130 mL

For signal reading, an HTRF®-Certified Reader is required. Make sure to use the set-up for Tb Cryptate. For a list of HTRF-compatible readers and setup recommendations, please visit our website at: www.revvity.com

For HTRF microplate recommendations, please visit www.revvity.com

STORAGE AND STABILITY

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

Tagged KRAS G12C & SOS1, GTP, and anti-Tag stock solutions can be frozen and thawed only twice. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at ≤-60°C.

Thawed Binding domain detection buffer #2 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 Binding domain detection buffer #2. The use of an incorrect buffer 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 the buffer. It is recommended to filter this buffer 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 premixed prior to dispensing.
- Compounds must be prepared in Binding domain detection buffer #2. We recommend keeping DMSO below 1% during the assay (20 µL final volume).

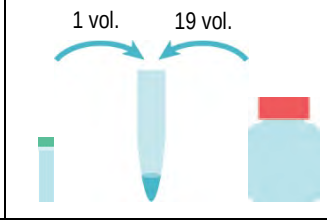
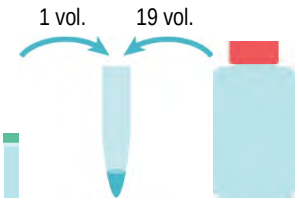
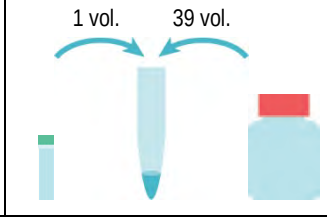
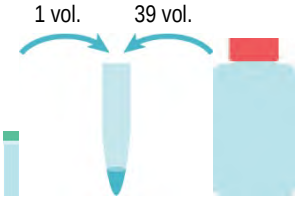
TO PREPARE STOCK SOLUTIONS:

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-KRAS G12C			
Thaw the Tag1-KRAS G12C Mix gently. This 20X stock solution can be frozen and stored at ≤ -60°C.			Thaw the Tag1-KRAS G12C Mix gently. This 20X stock solution can be frozen and stored at ≤ -60°C.
Tag2-SOS1			
Thaw the Tag2-SOS1. Mix gently. This 40X stock solution can be frozen and stored at ≤ -60°C.			Thaw the Tag2-SOS1. Mix gently. This 40X stock solution can be frozen and stored at ≤ -60°C.
KRAS/SOS1 Standard			
Thaw the KRAS/SOS1 standard. Mix gently. This standard stock solution can be frozen and stored at ≤ -16°C.			Thaw the KRAS/SOS1 standard. Mix gently. This standard stock solution can be frozen and stored at ≤ -16°C.
Anti-Tag1 XL665 antibody			
Thaw the Anti-Tag1 XL665 antibody. Mix gently. This 20X XL665 stock solution can be frozen and stored at ≤ -60°C.			Thaw the Anti-Tag1 XL665 antibody. Mix gently. This 20X XL665 stock solution can be frozen and stored at ≤ -60°C.
Anti-Tag2 Terbium cryptate antibody			
Thaw the Anti-Tag2 Terbium cryptate antibody. Mix gently. This 50X Terbium stock solution can be frozen and stored at ≤ -60°C.			Thaw the Anti-Tag2 Terbium cryptate antibody. Mix gently. This 50X Terbium stock solution can be frozen and stored at ≤ -60°C.
Binding Domain detection buffer #2			
Thaw the Binding domain detection buffer #2. The thawed buffer can be stored at 2-8°C on your premises.			Thaw the Binding domain detection buffer #2. The thawed buffer can be stored at 2-8°C on your premises.
KRAS/SOS1 GTP			
Thaw the KRAS/SOS1 GTP. Mix gently. This 40X GTP stock solution can be frozen and stored at ≤ -16°C.			Thaw the KRAS/SOS1 GTP. Mix gently. This 40X GTP stock solution can be frozen and stored at ≤ -16°C.

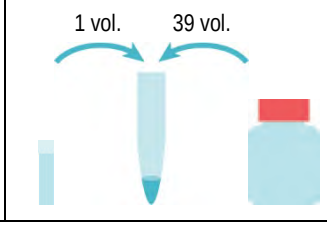
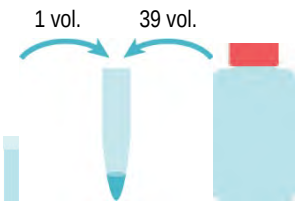
TO PREPARE TAG1- KRAS G12C AND TAG2- SOS1 WORKING SOLUTIONS:

Each well requires 4 µL of Tag2-protein and 2µL of Tag1-protein (or see section below to prepare the pre-mixed Tag1-KRAS G12C + GTP working solutions):

500 TESTS		10,000 TESTS	
Tag1-KRAS G12C			
Dilute 20-fold the stock solution (thawed reagent) of Tag1-KRAS G12C with Binding domain detection buffer #2: e.g. 10 µL of Tag1-KRAS G12C stock solution + 190 µL of Binding domain detection buffer #2.			Dilute 20-fold the stock solution (thawed reagent) of Tag1-KRAS G12C with Binding domain detection buffer #2: e.g. 1 mL of Tag1-KRAS G12C stock solution + 19 mL of Binding domain detection buffer #2.
Tag2-SOS1			
Dilute 40-fold the stock solution (thawed reagent) of Tag2-SOS1 with Binding domain detection buffer #2: e.g. 10 µL of Tag2-SOS1 stock solution + 390 µL of Binding domain detection buffer #2.			Dilute 40-fold the stock solution (thawed reagent) of Tag2-SOS1 with Binding Domain detection buffer #2: e.g. 1 mL of Tag2-SOS1 stock solution + 39 mL of Binding domain detection buffer #2.

TO PREPARE GTP WORKING SOLUTIONS:

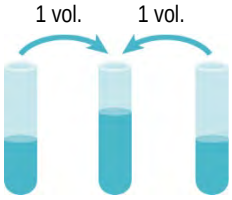
Each well requires 2 µL of GTP (or see section below to prepare the pre-mixed Tag1-KRAS G12C + GTP working solutions):

500 TESTS		10,000 TESTS	
KRAS/SOS1 GTP			
Dilute 40-fold the stock solution (thawed reagent) of GTP with Binding domain detection buffer #2: e.g. 10 µL of GTP stock solution + 390 µL of Binding domain detection buffer #2.			Dilute 40-fold the stock solution (thawed reagent) of GTP with Binding domain detection buffer #2: e.g. 1 mL of GTP stock solution + 39 mL of Binding domain detection buffer #2.

TO PREPARE PRE-MIXED TAG1-KRAS G12C/GTP WORKING SOLUTIONS:

Each well requires 4 µL of pre-mixed Tag1-KRAS G12C + GTP:

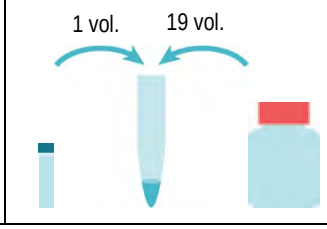
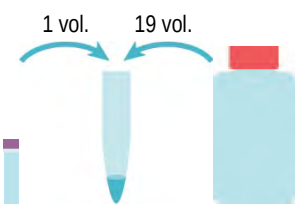
It is mandatory to mix Tag1-KRAS G12C with GTP just prior dispensing to avoid alteration of signal.

500 TESTS		10,000 TESTS
Tag1-KRAS G12C + KRAS/SOS1 GTP		
Dilute 40-fold the stock solution (thawed reagent) of GTP & 20-fold the stock solution (thawed reagent) of Tag1-KRAS G12C with Binding domain detection buffer #2. Then pre-mix the two ready-to-use solutions just prior to dispensing the reagents: e.g. 0.5mL of GTP + 0.5mL of Tag1-KRAS G12C protein		Dilute 40-fold the stock solution (thawed reagent) of GTP & 20-fold the stock solution (thawed reagent) of Tag1-KRAS G12C with Binding domain detection buffer #2. Then pre-mix the two ready-to-use solutions just prior to dispensing the reagents: e.g. 3mL of GTP + 3mL of Tag1-KRAS G12C protein

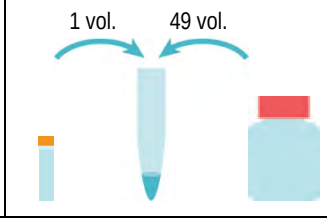
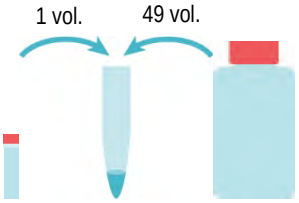
TO PREPARE ANTI-TAG1 XL665 ANTIBODY AND ANTI-TAG2 TB CRYPTATE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL of each anti-Tag donor & acceptor reagent.

<table>
<tr><th colspan="2">500 TESTS</th><th colspan="2">10,000 TESTS</th></tr>
<tr><td colspan="4">Anti-Tag1 XL665 antibody</td></tr>
<tr><td>Dilute 20-fold the stock solution (thawed reagent) of Anti-Tag1 XL665 antibody with Binding domain detection buffer #2:

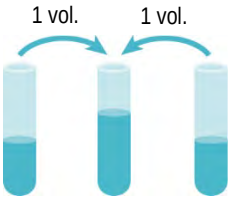
e.g. 10 µL of Anti-Tag1 XL665 antibody stock solution + 190 µL of Binding domain detection buffer #2.</td><td> 
</td><td> 
</td><td>Dilute 20-fold the stock solution (thawed reagent) of Anti-Tag1 XL665 antibody with Binding domain detection buffer #2:

e.g. 1 mL of Anti-Tag1 XL665 antibody stock solution + 19 mL of Binding domain detection buffer #2.</td></tr>
<tr><td colspan="4">Anti-Tag2 Tb cryptate antibody</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag2 Tb cryptate antibody with Binding domain detection buffer #2:

e.g. 50 µL of Anti-Tag2 Tb cryptate antibody stock solution + 2450 µL of Binding domain detection buffer #2.</td><td> 
</td><td> 
</td><td>Dilute 50-fold the 50X stock solution (thawed reagent) of Anti-Tag2 Tb cryptate antibody with Binding domain detection buffer #2:

e.g. 1 mL of Anti-Tag2 Tb cryptate antibody stock solution + 49 mL of Binding domain detection buffer #2.</td></tr>
<tr><td colspan="4">anti-Tag HTRF detection solutions (pre-mixed)</td></tr>
<tr><td colspan="2">Pre-mix the two ready-to-use anti-Tag HTRF detection solutions just prior to dispensing the reagents:

e.g. 2.5 mL of Anti-Tag1 XL665 antibody +

2.5 mL of Anti-Tag2 Tb cryptate antibody</td><td> 
</td><td>Pre-mix the two ready-to-use anti-Tag HTRF detection solutions just prior to dispensing the reagents:

e.g. 20 mL of Anti-Tag1 XL665 antibody +

20 mL of Anti-Tag2 Tb cryptate antibody</td></tr>
</table>

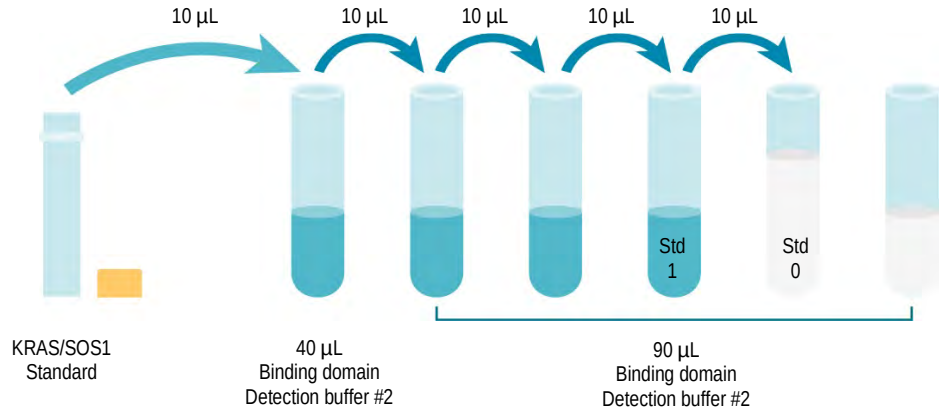
TO PREPARE WORKING KRAS/SOS1 STANDARD SOLUTIONS:

- Each well requires 2 µL of standard.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

- Dilute the KRAS/SOS1 standard stock solution 5-fold with Binding domain detection buffer #2 to prepare high standard (Std 7): Take 10 µL of standard stock solution and add it to 40 µL of Binding domain detection buffer #2. Mix gently.
- Use the high standard (Std 7) to prepare the standard curve using 10-fold serial dilutions, as follows:
  - Dispense 90 µL of Binding domain detection buffer #2 into each vial from Std 6 to Std 0
  - Add 10 µL of standard to 90 µL of Binding domain detection buffer #2, mix gently, and repeat the serial dilution to make the other standard solutions: std6, std5, std4, std3, std2, std1

This will create 7 standards for the analyte. Std 0 is Binding domain detection buffer #2.



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS	FINAL CONCENTRATIONS
Standard Stock solution	Thawed stock solution	50 000 µM	
Standard 7	10 µL standard stock solution + 40 µL Binding domain detection buffer #2	10 000 µM	1 000 µM
Standard 6	10 µL Standard 7 + 90 µL Binding domain detection buffer #2	1 000 µM	100 µM
Standard 5	10 µL Standard 6 + 90 µL Binding domain detection buffer #2	100 µM	10 µM
Standard 4	10 µL Standard 5 + 90 µL Binding domain detection buffer #2	10 µM	1 µM
Standard 3	10 µL Standard 4 + 90 µL Binding domain detection buffer #2	1 µM	0.1 µM
Standard 2	10 µL Standard 3 + 90 µL Binding domain detection buffer #2	0.1 µM	0.01 µM
Standard 1	10 µL Standard 2 + 90 µL Binding domain detection buffer #2	0.01µM	0.001µM
Standard 0	90 µL Binding domain detection buffer #2	0	0

ASSAY MANUAL

	STANDARD	SAMPLES
Step 1 	Into each standard well, dispense: 2 µL of standard, 4 µL of premixed Tag1-KRAS G12C + GTP, 4 µL of Tag2-SOS1	Into each sample well, dispense: 2 µL of compound or buffer, 4 µL of premixed Tag1-KRAS G12C + GTP, 4 µL of Tag2-SOS1
Step 2 	Into all standard & sample wells, dispense 10 µL of pre-mixed Anti-Tag1 XL665 antibody and Anti-Tag2 Tb cryptate antibody.	
Step 3 	Seal the plate and incubate for 2 hours at room temperature.	
Step 4 	Remove the plate sealer and read on an HTRF® compatible reader.	

EXAMPLE OF PLATE MAP

	1	2	3	4	5	6
A	Buffer control: 20 µL Binding domain detection buffer #2	Repeat Well A1	Repeat Well A1	Vehicle control: 2 µL vehicle 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well A4	Repeat Well A4
B	Negative control: 10µL Binding domain detection buffer #2 10 µL pre-mixed anti-Tag reagents	Repeat Well B1	Repeat Well B1	Compound...: 2 µL Compound 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well B4	Repeat Well B4
C	Std 0: 2 µL Standard 0 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well C1	Repeat Well C1	Compound...: 2 µL Compound 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well C4	Repeat Well C4
D	Std 1: 2 µL Standard 1 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well D1	Repeat Well D1	Compound...: 2 µL Compound 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well D4	Repeat Well D4
E	Std 2: 2 µL Standard 2 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well E1	Repeat Well E1	Compound...: 2 µL Compound 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well E4	Repeat Well E4
F	Std 3: 2 µL Standard 3 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well F1	Repeat Well F1	Compound...: 2 µL Compound 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well F4	Repeat Well F4
G	Std 4: 2 µL Standard 4 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well G1	Repeat Well G1	Compound...: 2 µL Compound 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well G4	Repeat Well G4
H	Std 5: 2 µL Standard 5 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well H1	Repeat Well H1			
I	Std 6: 2 µL Standard 6 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well I1	Repeat Well I1			
J	Std 7: 2 µL Standard 7 4 µL Tag1-KRAS G12C + GTP 4 µL Tag2-SOS1 10 µL pre-mixed anti-Tag reagents	Repeat Well J1	Repeat Well J1			

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.

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

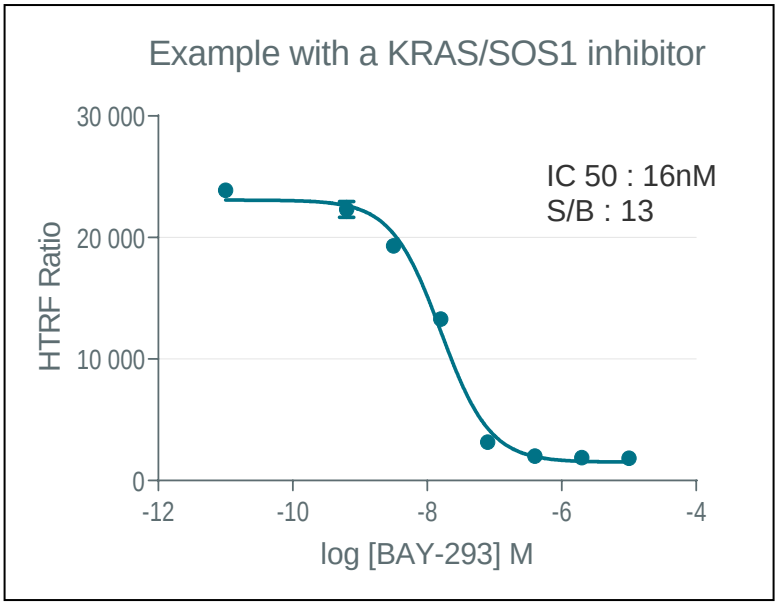
For more information about data reduction, please visit www.revvity.com

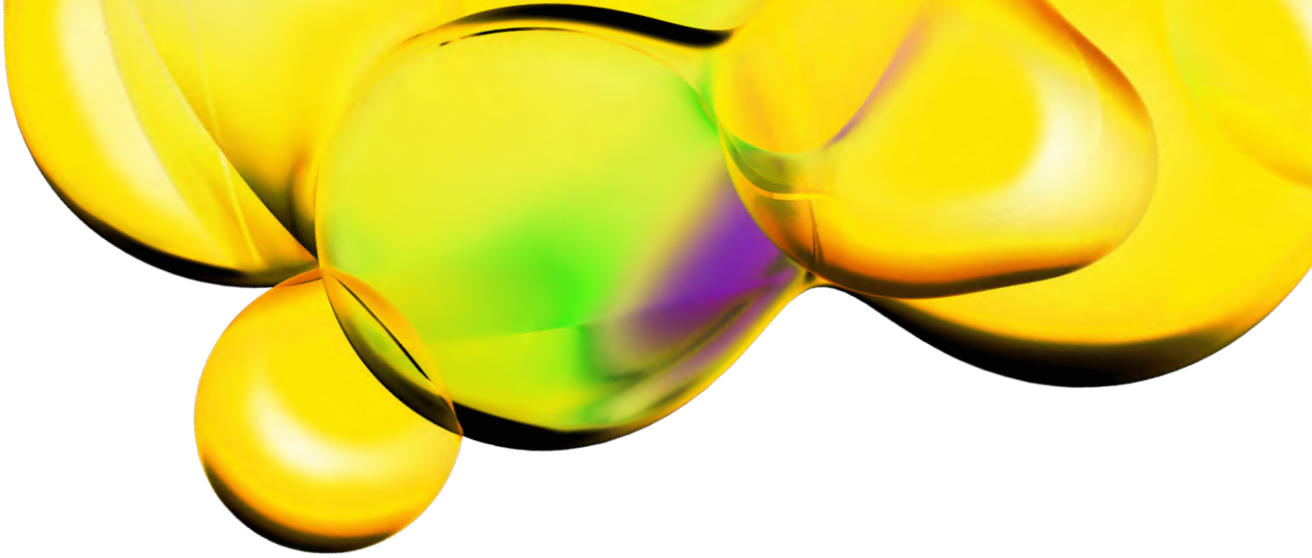
RESULTS

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

Readouts obtained on an HTRF compatible reader with a flash lamp.

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





Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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