Aurora Biolabs
High Purity Protein for Research

Kras (G12C) Nucleotide Exchange Assay Kit

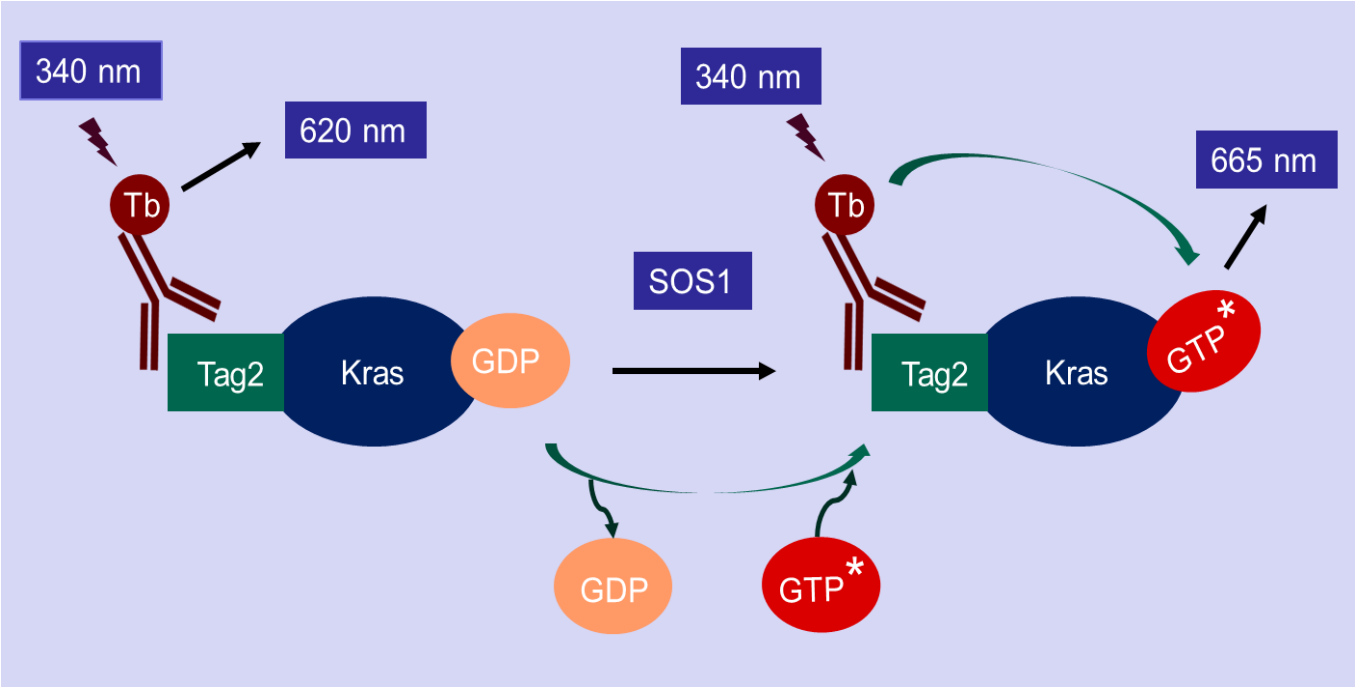
Catalog Number: 5727-4122NK

Background

Kras is a member of the RAS protein family, which are a class of small GTPases involved in cell signaling pathways. The Ras signaling pathway plays an important role in cell proliferation and differentiation. Conversion of Kras from the inactive GDP-bound state to the active GTP-bound state triggers the downstream effector and promotes cell growth. *RAS* genes are frequently mutated in various human tumors. These mutations block the GTPase activity of RAS and lock RAS in the GTP-bound state, resulting in constitutively active signals through the downstream cascades leading to cancer cell proliferation.

Assay Principle

The Kras (G12C) nucleotide exchange assay is a TR-FRET based assay. The assay kit is designed to detect the GTP binding status of Kras (G12C) in the presence of SOS1, the most-studied guanine nucleotide exchange factor (GEF) of Kras. The Tag2-Kras in this assay kit is recognized by a Terbium-labeled anti-Tag2 antibody (HTRF donor). If Kras binds to a fluorescence-labeled GTP (HTRF acceptor), the donor and the acceptor will be brought in close proximity. Excitation of Terbium (340 nm) generates fluorescence resonance energy transfer (FRET) to the fluorescence-labeled GTP acceptor, which consequently fluoresces at 665 nm (figure below). Thus, GTP binding to Kras can be quantitively measured by calculation of the fluorescent ratio of 665 nm/620 nm. The inhibitor blocking the nucleotide exchange will reduce the HTRF signal.



Aurora Biolabs, LLC; www.aurorabiolabs.com; order@aurorabiolabs.com; info@aurorabiolabs.com
Tel: 858-214-4510 or 858453-5700 Fax: 855-898-3979

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Application

High throughput screening of compounds that inhibit Kras activation for drug discovery.

Plate Reader

A HTRF® certified microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is required.

Components

Catalog number	Item	Amount	Storage
5727-NK-B	2x Assay Buffer	25 mL	-20°C
5727-4122-T2	Recombinant human Tag2-Kras (G12C), GDP loaded	5 µL	-80°C
7671	Recombinant human SOS1	5 µL	-80°C
48735	GTP, fluorescence labeled	100 µL	-80°C
37882	Anti-Tag2 antibody, Tb labeled	20 µL	-80°C
	384-well microplate	1	Room temperature

Materials needed but not supplied

1. Microplate reader, HTRF® certified microplate reader
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

Assay Protocol A

If you screen compounds that inhibit the interaction of Kras (G12C) with SOS1, follow the assay protocol A. If you look for the compounds that bind to Kras (G12C) and inhibit the GTP exchange, go to assay protocol B.

1. Prepare 1X assay buffer containing 1 mM DTT (1X DTT-containing assay buffer)
   For example, mix 996 µl distilled water with 1000 µl of 2X assay Buffer (catalogue number: 5727-NK-B) and 4 µl of 0.5 M DTT. Make only enough 1X DTT-containing assay buffer as needed for the assay. Store the remaining 2X assay buffer at -20°C.
2. Prepare the inhibitor compound solution
   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 2 µl to the 20 µl reaction).
   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare as series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).
3. Prepare SOS1 solution
   Thaw SOS1 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted enzyme at -80°C.
   Note: SOS1 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.
   Dilute the SOS1 protein 1,000-fold (1 µL SOS1 + 999 µL 1X DTT-containing assay buffer).
   Add 4 µl of diluted protein solution to each of positive control well and inhibitor test well.
   Add 4 µl of 1X DTT-containing assay buffer to each of negative control well.
4. Add inhibitor
   Add 2 µl of diluted compound solution to each inhibitor test well.
   Add 2 µl of inhibitor solvent solution to each of negative control well.
   Incubate at room temperature for 30 minutes (optional).

5. Prepare Kras solution

   Thaw Kras protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C.

   Note: Kras protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the Kras protein 380-fold (1 µL Kras G12C + 379 µL 1X DTT-containing assay buffer).

   Add 4 µl of diluted protein solution to each well.

6. Prepare dye solution

   Dilute Terbium-labeled anti-Tag2 antibody 1:200 and dilute fluorescence-labeled GTP 1:40 in 1X DTT-containing assay buffer. For example: 1 µl of Terbium-labeled anti-Tag2 antibody + 5 µl of fluorescence-labeled GTP + 194 µl of 1X DTT-containing assay buffer.

   Add 10 µl of this dye mixture to each well.

7. Incubate the reaction at room temperature for 20 minutes.

8. Measure fluorescent intensity

   HTRF compatible microplate reader is needed to measure fluorescent intensity of the samples. Fluorescent intensity should be measured twice:

   1. Excitation wavelength at 340 nm and emission at 620 nm.
   2. Excitation wavelength at 340 nm and emission at 665 nm.

Protocol Summary

Component	Background	Positive Control	Inhibitor Test
1X DTT-containing assay buffer	4 µl		
SOS1 protein		4 µl	4 µl
Inhibitor solvent	2 µl	2 µl	
Inhibitor solution			2 µl
	6 µl	6 µl	6 µl
Incubate at room temperature for 30 minutes.			
1X DTT-containing assay buffer	4 µl		
Kras G12C protein		4 µl	4 µl
Tb-anti Tag2 + GTP solution	10 µl	10 µl	10 µl
Total Volume	20 µl	20 µl	20 µl
Incubate at room temperature for 20 minutes.			

Data Analysis

1. Calculate the ratio of the fluorescent intensity of each well.

$$Ratio1 = \frac{\text{Fluorescent intensity at 620 nm}}{\text{Fluorescent intensity at 340 nm}}$$

2. Calculate the ratio of the fluorescent intensity of each well.

$$Ratio2 = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 340 nm}}$$

3. Calculate sample signal.

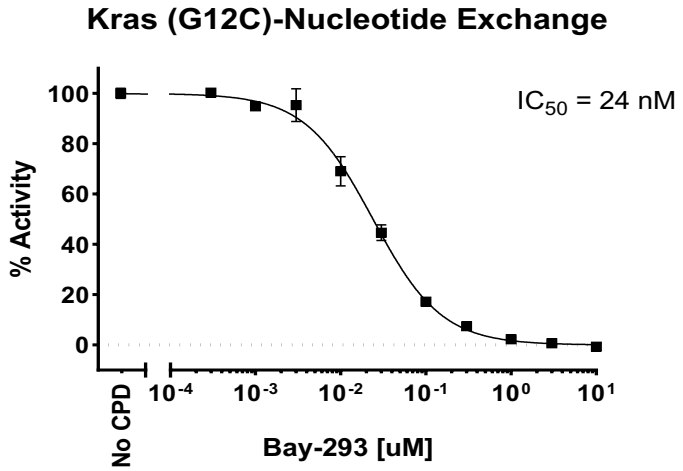
$$Sample\ signal = \frac{Ratio2}{\text{Ratio1}}$$

4. Calculate percentage activity

   In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\%\ \text{activity} = \frac{S - N}{\text{P} - \text{N}} X100$$

Data Presentation



Assay Protocol B

If you screen of the compounds that inhibit the interaction of Kras (G12C) with SOS1, go to assay protocol A. If you look for the compounds that bind to Kras (G12C) and inhibit the GTP exchange, follow the assay protocol B.

1. Prepare 1X aasay buffer containing 1 mM DTT (1X DTT-containing assay buffer)
   For example, mix 996 µl distilled water with 1000 µl of 2X assay Buffer (catalogue number: 5727-NK-B) and 4 µl of 0.5 M DTT. Make only enough 1X DTT-containing assay buffer as needed for the assay. Store the remaining 2X assay buffer at -20°C.
2. Prepare the inhibitor compound solution
   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 2 µl to the 20 µl reaction).
   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare as series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).
3. Prepare Kras solution
   Thaw Kras protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted enzyme at -80°C.
   Note: Kras protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.
   Dilute the Kras protein 380-fold (1 µL Kras G12C + 379 µL 1X DTT-containing assay buffer).
   Add 4 µl of diluted protein solution to each of positive control well and inhibitor test well.
   Add 4 µl of 1X DTT-containing assay buffer to each of negative control well.
4. Add inhibitor
   Add 2 µl of diluted compound solution to each inhibitor test well.
   Add 2 µl of inhibitor solvent solution to each of negative control well.
   Incubate at room temperature for 30 minutes (optional).

5. Prepare SOS1 solution

   Thaw SOS1 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted protein at -80°C.

   Note: SOS1 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the SOS1 protein 400-fold (1 µL SOS1 + 399 µL 1X DTT-containing assay buffer).

   Add 4 µl of diluted protein solution to each well.

6. Prepare dye solution

   Dilute Terbium-labeled anti-Tag2 antibody 1:200 and dilute fluorescence-labeled GTP 1:40 in 1X DTT-containing assay buffer. For example: 1 µl of Terbium-labeled anti-Tag2 antibody + 5 µl of fluorescence-labeled GTP + 194 µl of 1X DTT-containing assay buffer.

   Add 10 µl of this dye mixture to each well.

7. Incubate the reaction at room temperature for 20 minutes.

8. Measure fluorescent intensity

   HTRF compatible microplate reader is needed to measure fluorescent intensity of the samples. Fluorescent intensity should be measured twice:

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Inhibitor solvent	2 µl	2 µl	
Inhibitor solution			2 µl
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Incubate at room temperature for 30 minutes.			
1X DTT-containing assay buffer	4 µl		
SOS1 protein		4 µl	4 µl
Tb-anti Tag2 + GTP solution	10 µl	10 µl	10 µl
Total Volume	20 µl	20 µl	20 µl
Incubate at room temperature for 20 minutes.			

Data Analysis

9. Calculate the ratio of the fluorescent intensity of each well.

$$Ratio1 = \frac{\text{Fluorescent intensity at 620 nm}}{\text{Fluorescent intensity at 340 nm}}$$

10. Calculate the ratio of the fluorescent intensity of each well.

$$Ratio2 = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 340 nm}}$$

11. Calculate sample signal.

$$Sample\ signal = \frac{Ratio2}{\text{Ratio1}}$$

12. Calculate percentage activity

In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\%\ \text{activity} = \frac{S - N}{\text{P} - \text{N}} X100$$

Data Presentation