Kras (WT) Nucleotide Exchange Assay Kit

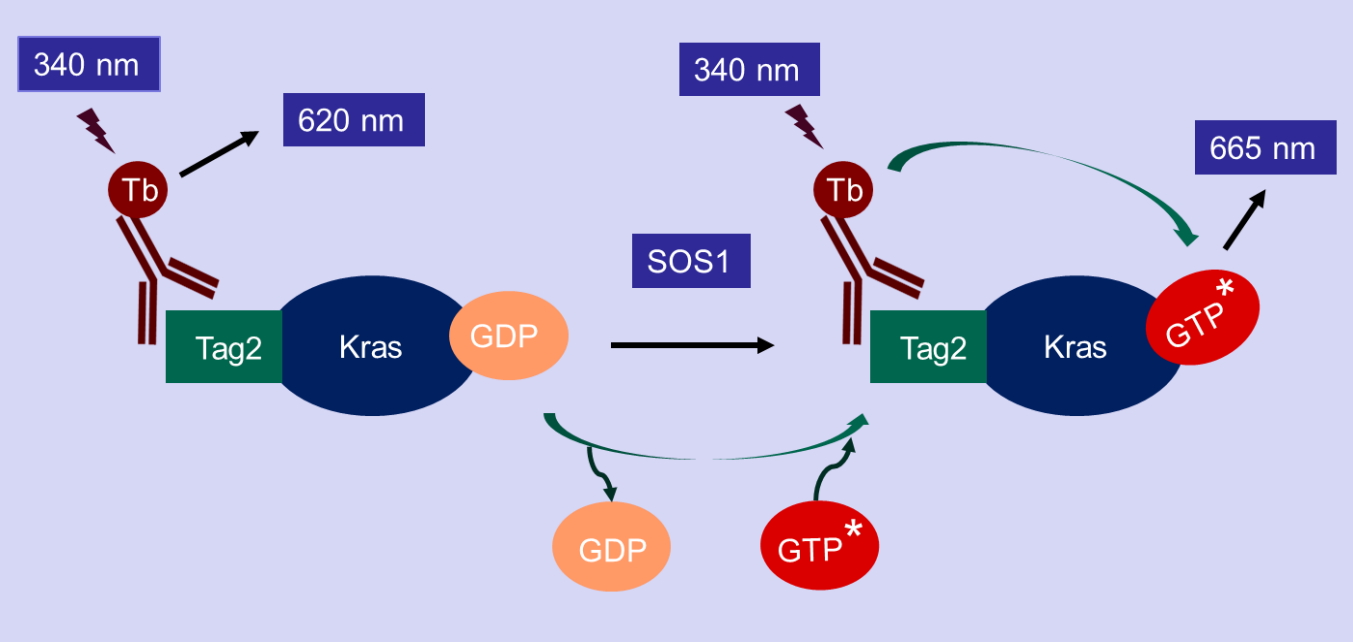
Catalog Number: 5727-4121NK

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 (wild type, WT) nucleotide exchange assay is a TR-FRET based assay. The assay kit is designed to detect the GTP binding status of wild type Kras 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.



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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
5727-4121-T2	Recombinant human Tag2-Kras (WT), GDP loaded	10 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 (such as Tecan M1000 or Tecan Spark, etc.)
2. 0.5 M DTT
3. Adjustable micro-pipettor
4. Sterile Tips

Assay protocol

This protocol is designed to screen inhibitors that block interaction between Kras (WT) and SOS1.

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 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 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 and positive 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 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 to 210-fold (1 µL Kras WT + 209 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
Subtotal Volume	6 µl	6 µl	6 µl
Incubate at room temperature for 30 minutes.			
1X DTT-containing assay buffer	4 µl		
Kras G12V 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 HTRF signal (ratio of the fluorescent intensity at 665 mm/620 mm) of each well.

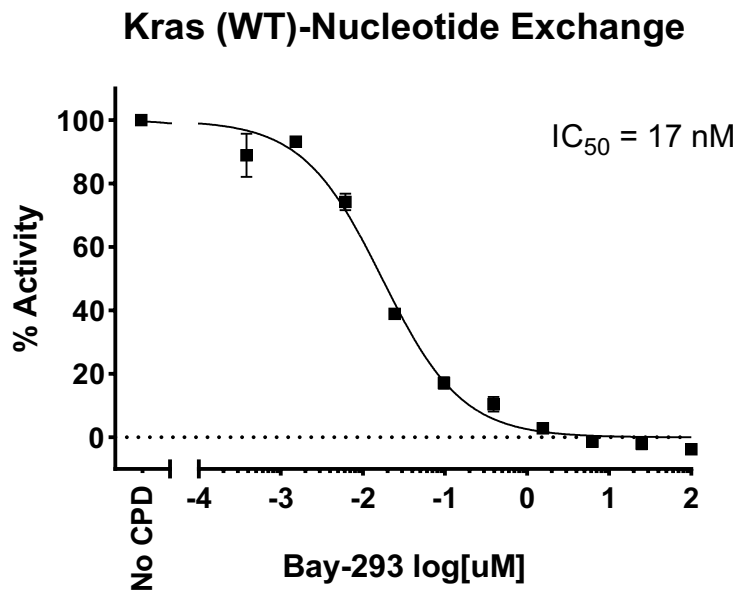
$$HTRF = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 620 nm}} X10{,}000$$

2. 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



Related products:

Product Name	Catalog #	Size
Kras Wild Type (WT), GST-tag	5727-4121G	50 µg, 100 µg
Kras WT, GST-tag, GDP Loaded	5727-WTG-G	50 µg, 100 µg
Kras WT, GST-tag, GppNHp loaded	5727-WTG-GP	50 µg, 100 µg

Kras G12C, His -tag	5727-4122H	50 µg, 100 µg
Kras G12C, GST-tag	5727-4122G	50 µg, 100 µg
Kras G12C, GST-tag, GDP Loaded	5727-4122G -G	50 µg, 100 µg
Kras G12C, GST-tag, GppNHp loaded	5727-4122G -GP	50 µg, 100 µg
Kras G12D, GST-tag	5727-4123G	50 µg, 100 µg
Kras G12D, GST-tag, GDP Loaded	5727-4123G -G	50 µg, 100 µg
Kras G12D, GST-tag, GppNHp loaded	5727-4123G -GP	50 µg, 100 µg
Kras G12R, GST-tag,	5727-4127G	50 µg, 100 µg
Kras G12R, GST-tag, GDP Loaded	5727-4127G -G	50 µg, 100 µg
Kras G12R, GST-tag, GppNHp loaded	5727-4127G -GP	50 µg, 100 µg
Kras G12V, GST-tag,	5727-4128G	50 µg, 100 µg
Kras G12V, GST-tag, GDP Loaded	5727-4128G -G	50 µg, 100 µg
Kras G12V, GST-tag, GppNHp loaded	5727-4128G -GP	50 µg, 100 µg
Kras WT Nucleotide Exchange Assay Kit	5727-4121NK	384 reactions
Kras G12C Nucleotide Exchange Assay Kit	5727-4122NK	384 reactions
Kras G12D Nucleotide Exchange Assay Kit	5727-4123NK	384 reactions
Kras G12R Nucleotide Exchange Assay Kit	5727-4127NK	384 reactions
Kras G12V Nucleotide Exchange Assay Kit	5727-4128NK	384 reactions
Kras WT – cRAF Binding Assay Kit	5727-4121BK	384 reactions
Kras G12C – cRAF Binding Assay Kit	5727-4122BK	384 reactions
Kras G12D– cRAF Binding Assay Kit	5727-4123BK	384 reactions
Kras G12R– cRAF Binding Assay Kit	5727-4127BK	384 reactions
Kras G12V – cRAF Binding Assay Kit	5727-4128BK	384 reactions
Human RBD-RAF1, N-His tag, C-FLAG tag	7237231	50 µg, 100 µg
Human SOS1, No tag	7671	50 µg, 100 µg
Human SOS1, Avi-His tag	7671HA	50 µg, 100 µg

Products are for research use only and are not intended for human use. We do not sell to patients.