Aurora Biolabs
High Purity Protein for Research

Kras (G12D)-cRAF Binding Assay Kit

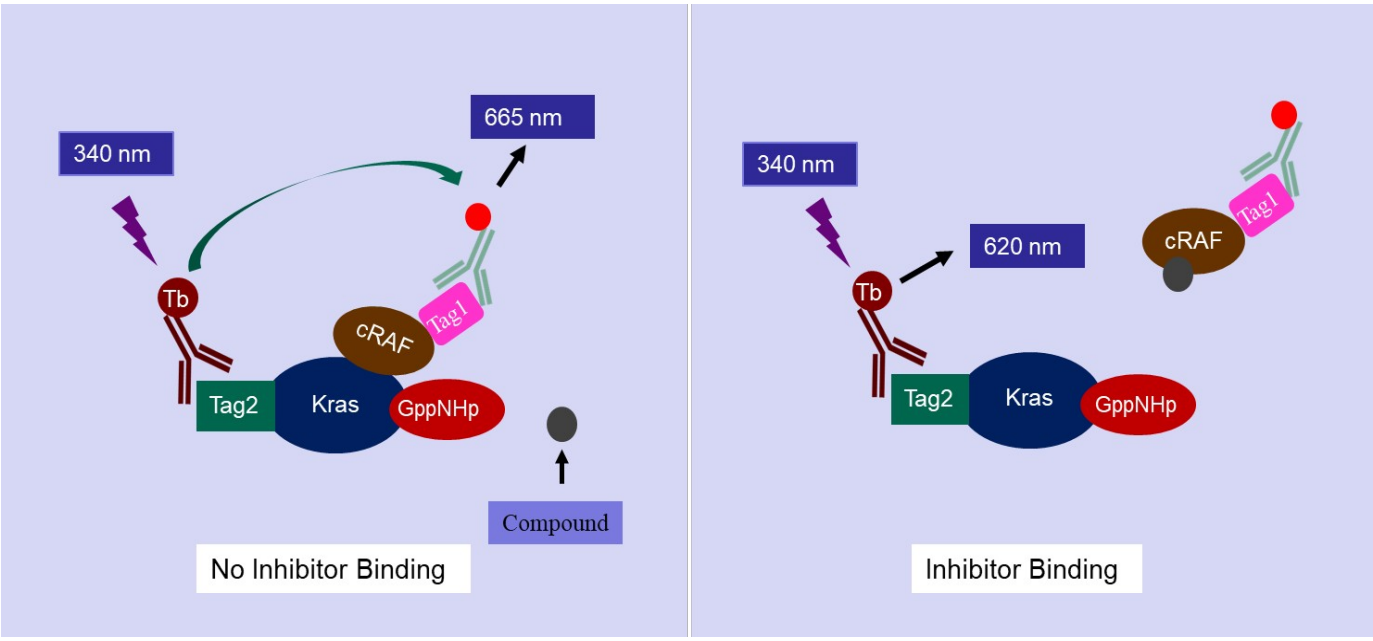
Catalog Number: 5727-4123BK

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 regulates diverse cellular processes, including cell proliferation, differentiation, and survival. Conversion of Ras from the inactive GDP-bound state to the active GTP-bound state activates the downstream effector and promotes cell growth. RAF is a key downstream effector of RAS. Since the frequently mutated Ras genes are associated with various human tumors, the Ras-RAF signaling pathway is considered a potential therapeutic target for cancer treatment.

Assay Principle

The Kras (G12D)-cRAF binding assay kit is a TR-FRET based assay, which is designed to detect the binding status between Kras and cRAF. Tag2-Kras (G12D) in this assay kit is loaded with GppNHp, which represents the activated Kras. The Ras binding domain (RBD) of cRAF has a Tag1 at N-terminus. A Terbium-labeled anti-Tag2 antibody binding to the Tag2-Kras serves as a fluorescence donor (HTRF donor), activation of which results in fluorescence resonance energy transfer (FRET) if Tag1-cRAF binds to the Kras, since the binding brings Terbium on the anti-Tag2 antibody close to the fluorophore on the anti-Tag1 antibody (HTRF acceptor). Thus, the binding status can be quantitively measured by calculating the ratio of the emission fluorescence intensity of the acceptor (665 nm) and donor (620 nm). Blocking the Kras-cRAF binding will reduce the HTRF signal.



Kras (G12D)-cRAF Binding Assay Kit

Catalog Number: 5727-4123BK

Application

High throughput screening of compounds that inhibit the binding between activated Kras (G12D) and cRAF 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-BK-B	Binding buffer	25 mL	-20°C
7237231-T1	Recombinant human Tag1-cRAF, RBD	5 µL	-80°C
5727-4123-T2P	Recombinant human Tag2-Kras (G12D), GppNHp loaded	10 µL	-80°C
37882	Terbium-labeled anti-Tag2 antibody	20 µL	-80°C
44732	Fluorescence labeled anti-Tag1 antibody	100 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

1. Prepare Binding buffer containing 1 mM DTT (DTT containing Binding buffer)
   For example, mix 998 µl of binding Buffer and 2 µl of 0.5 M DTT. Make only enough DTT-containing Binding buffer as needed for the assay. Store the remaining Binding 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 Binding 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 Binding 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 Binding buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).
3. Prepare cRAF solution
   Thaw cRAF 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: cRAF protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.
   Dilute the cRAF protein 480-fold (1 µL cRAF + 479 µL DTT containing Binding buffer).
   Add 4 µl of diluted protein solution to each positive control well and inhibitor test well.
   Add 4 µl of DTT containing Binding 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 (G12D) 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 175-fold (1µL Kras G12D + 174 µL DTT containing Binding 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 anti-Tag1 antibody 1:40 in DTT containing Binding buffer. For example: 1 µl of Terbium-labeled anti-Tag2 antibody + 5 µl of fluorescence-labeled anti-Tag1 antibody + 194 µl of DTT containing Binding buffer.

   Add 10 µl of this dye mixture to each well.

7. Incubate the reaction at room temperature for 30 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	Negative Control	Positive Control	Inhibitor Test
DTT containing Binding buffer	4 µl		
cRAF 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.			
Kras (G12D) protein	4 µl	4 µl	4 µl
Dye solution	10 µl	10 µl	10 µl
Total Volume	**20 µl**	**20 µl**	**20 µl**
Incubate at room temperature for 30 minutes.			

Data Analysis

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

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

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

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

3. Calculate sample signal.

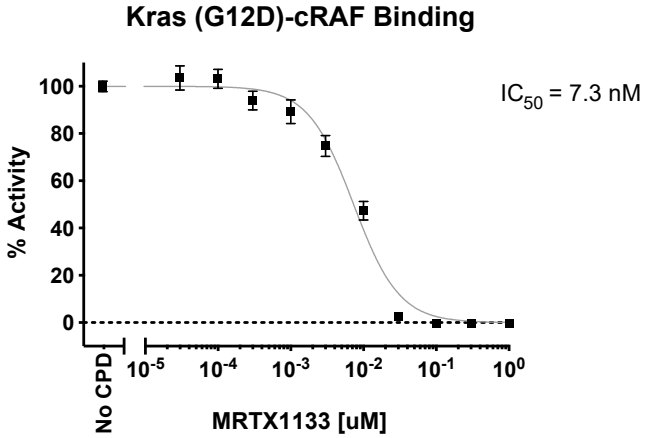
$$Sample\ signal = \frac{Ratio2}{\text{Ratio1}}$$

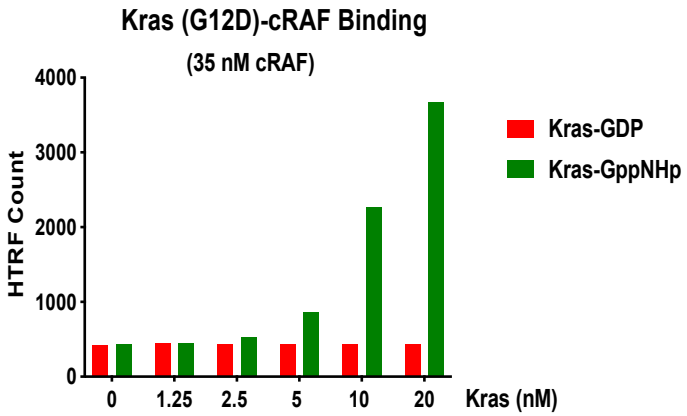
4. Calculate percentage activity

   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.

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

Assay result





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.