c-RAF (Y340D, Y341D) Kinase Assay Kit

Description

The c-RAF (Y340D, Y341D) Kinase Assay Kit is designed to measure c-Raf (Raf proto-oncogene serine/threonine-protein kinase) mutant (Y340D, Y341D) kinase activity for screening and profiling applications using Kinase-Glo™ MAX as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant c-RAF kinase mutant (Y340D, Y341D) (amino acids 306-648(end)), kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

c-RAF, also known as Raf-1 or proto-oncogene c-Raf, is a member of the Raf (rapidly accelerated fibrosarcoma) kinase family. C-Raf is ubiquitously expressed and seems to be essential for embryogenesis and survival. It plays a role in cell proliferation, differentiation, apoptosis, and senescence. It phosphorylates MEK1/2 (MAP kinase kinase 1/2), resulting in ERK1/2 (extracellular signal-regulated kinase 1/2) activation. It is involved in cancer, by mediating cell proliferation and transformation. C-RAF is an attractive therapeutic target, and agents targeting this protein are currently being investigated in clinical trials for oncologic disorders.

Applications

Study enzyme kinetics and screen small molecular inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40008	c-RAF(Y340D, Y341D), GST-tag*	5 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	100 µl	-20°C
79569	5x RAF Substrate	1 ml	-80°C
79696	White 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

Name	Catalog #
Kinase-Glo™ MAX	Promega #V6071
DTT (Dithiothreitol), 1M, optional	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

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Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle

Kinase activity is measured using **Kinase-Glo™ Max** (Promega #V6071). The addition of the reagent results in the generation of a luminescent signal that correlates with the amount of ATP. The reagent is linear to 100 µM ATP.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Vemurafenib as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw **5x Kinase Buffer 1**, **500 µM ATP** and **5x RAF Substrate.**

   *Optional: If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.*

2. Prepare 3 ml of **1x Kinase Buffer 1** by mixing 600 µl of **5x Kinase Buffer 1** with 2400 µl of distilled water.

   *Note: 3 ml of **1x Kinase Buffer 1** is sufficient for 100 reactions.*

3. Prepare a **Master Mix** (25 µl/well): N wells x (6 µl of 5x Kinase Buffer 1 + 1 µl of 500 µM ATP + 10 µl of 5x RAF Substrate + 8 µl of distilled water).

4. Initiate the reaction by adding 25 µl of Master Mix to all wells.

5. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   3.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Kinase Buffer 1, 10-fold more concentrated than the desired final concentrations.

   For positive and negative controls, use 1x Kinase Buffer 1 (Diluent Solution).

   OR

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3.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

6. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

7. Add 5 µl of of Diluent Solution to the “Positive Control" and “Blank” wells.

8. Thaw **c-RAF kinase** on ice. Briefly spin the tube to recover its full content.

9. Dilute c-RAF to 2 ng/µl with 1x Kinase Buffer 1 (20 µl/ well).

10. Add 20 µl of diluted kinase to the wells designated “Positive Control” and "Test Inhibitor".

11. Add 20 µl of 1x Kinase Buffer 1 to the “Blank” wells.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x Kinase Buffer 1	20 µl	-	-
Diluted c-RAF (2 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

12. Incubate at 30°C for 45 minutes.

13. Thaw the Kinase-Glo™ MAX reagent.

14. At the end of the 45-minute reaction, add 50 µl of Kinase-Glo™ MAX reagent to each well.

15. Cover the plate with aluminum foil and incubate the plate at RT for 15 minutes.

16. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

17. The “Blank” value should be subtracted from all other readings.

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Reading Luminescence
Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

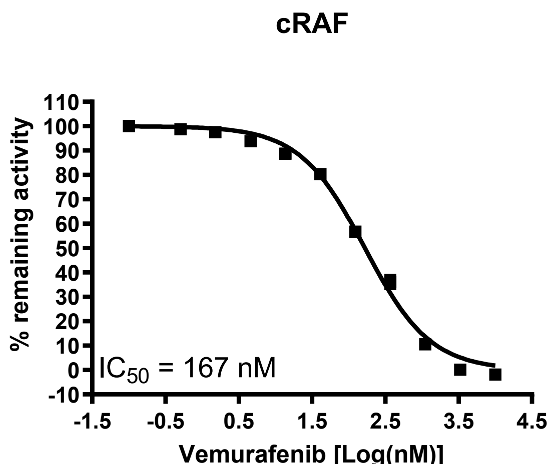

Figure 1: Inhibition of c-RAF (Y340D, Y341D) by Vemurafenib.
The inhibition of c-RAF (Y340D, Y341D) kinase activity was measured in the presence of increasing concentrations of Vemurafenib. The Blank value was subtracted from all other values. Results are expressed as percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Nakamura J.L., 2007 *Expert Opin. Ther. Targets* 11(4):463-472.

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Related Products

Products	*Catalog #*	*Size*
c-RAF, GST-Tag Recombinant	100519	100 µg/ 1 mg

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