6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
c-Raf Kinase Assay Kit
Catalog #79570

DESCRIPTION: c-Raf (Raf-1; proto-oncogen c-Raf) is a member of the Raf (rapidly accelerated fibrosarcoma) kinase family that plays an important role in regulating the MEK and ERK pathways. The *c-Raf Kinase Assay Kit* is designed for measuring c-Raf activity for screening and profiling applications. The *c-Raf Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant c-Raf enzyme, c-Raf substrate, ATP and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40008	c-Raf	5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
79569	5X Raf substrate	1 ml	-80°C.	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo MAX (Promega #V6071)
Dithiothreitol (DTT, 1 M; optional)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

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

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer, ATP** and **5x Raf substrate**.
(Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer**)

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2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 10 µl **5x Raf substrate** + 8 µl water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
5x Raf substrate	10 µl	10 µl	10 µl
Water	8 µl	8 µl	8 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
c-Raf (~2 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor”. For the “Positive Control" and “Blank”, add 5 µl of the same solution without inhibitor (Inhibitor buffer). *Note: Keep DMSO concentration of the Test Inhibitor at ≤10%, as final DMSO concentration in the reaction should be ≤1%.*

4) Prepare 3 ml of 1x Kinase assay buffer by mixing 600 µl of 5x Kinase assay buffer with 2400 µl water. 3 ml of 1x Kinase assay buffer is sufficient for 100 reactions.

5) To the wells designated as "Blank", add 20 µl of 1x Kinase assay buffer.

6) Thaw **c-Raf enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **c-Raf** required for the assay and dilute enzyme to ~2 ng/µl for **c-Raf** with 1x Kinase assay buffer. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **c-Raf enzyme** is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Initiate reaction by adding 20 µl of diluted **c-Raf** enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 45 minutes.

8) Thaw Kinase-Glo Max reagent.

9) After the 45 minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.

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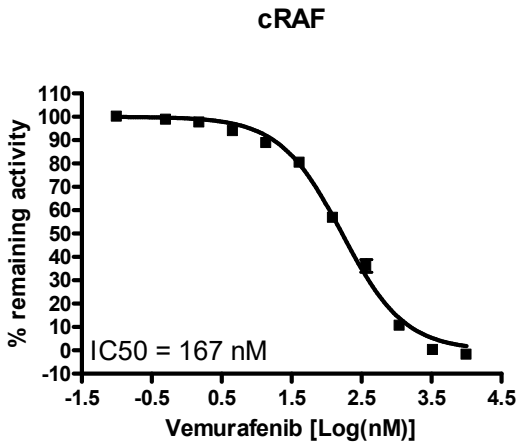
10) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, 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 are: 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 of Assay Results:



Inhibition of c-Raf enzyme by Vemurafenib, measured using the *c-Raf kinase assay kit* (Cat. #79570). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
cRAF (RAF1)	40008	10 µg
aRAF, His-tag	40010	10 µg
BRAF, GST-tag	40065	10 µg
BRAF/p50, FLAG-tag	40005	10 µg
B-Raf(V600E) Kinase Assay Kit	48688	96 rxns.
BRAF (V600E), GST-tag	40533	10 µg
BRAF, His-Tag (R444-DESC)	41237	10 µg