SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

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

B-Raf(V600E) Kinase Assay Kit

Catalog # 48688

DESCRIPTION: B-raf, after stimulation by activated RAS, phosphorylates MEK1 resulting in activation of the mitogen-activated protein (MAP) kinase cascade. Since the MAPK cascade has been shown to be a critical factor in melanoma development, it has been proposed that targeting B-raf could be a promising approach to combat melanoma. More importantly, 40-60% of melanomas display a mutation in BRAF, *e.g.* B-Raf(V600E), which leads to constitutive activation of the MAP kinase pathway. The *B-Raf(V600E) Kinase Assay Kit* is designed to measure B-Raf(V600E) kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The *B-Raf(V600E) Kinase Assay Kit* comes in a convenient 96-well format, with enough purified recombinant B-Raf enzyme, B-Raf substrate, ATP and Kinase Buffer 1 for 100 enzyme reactions. In addition, the *B-Raf(V600E) Kinase Assay Kit* includes wild type B-Raf enzyme as a positive control for researchers investigating B-Raf(V600E) specific inhibitors.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40533	B-Raf(V600E)	5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
40065	B-Raf(WT)*	1 µg	-80°C	
79334	5x Kinase Buffer 1	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.	

***** The kit contains enough B-Raf(WT) for about 15-20 wells.

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.

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

REFERENCES:

Cantwell-Dorris, ER, *et al. Mol Cancer Ther*. 2011 Mar;**10(3):**385-94
Obaid NM*, et al. Int J Mol Sci*. 2017 Mar 8;**18(3).** pii: E585.

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

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

2) Prepare the master mixture (25 µl per well): N wells x (6 µl 5x Kinase Buffer 1 + 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 Buffer 1	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
B-Raf(V600E) (~2 ng/µl) or B-Raf(WT) (2.5 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).

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

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

6) Thaw B-Raf(V600E) or B-Raf(WT) enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Calculate the amount of B-Raf required for the assay and dilute the enzyme to ~2 ng/µl for B-Raf(V600E), or 2.5 ng/µl for B-Raf(WT) with 1x Kinase Buffer 1. Store remaining undiluted enzyme in aliquots at -80°C. *Note: B-Raf enzymes are sensitive to*

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

freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

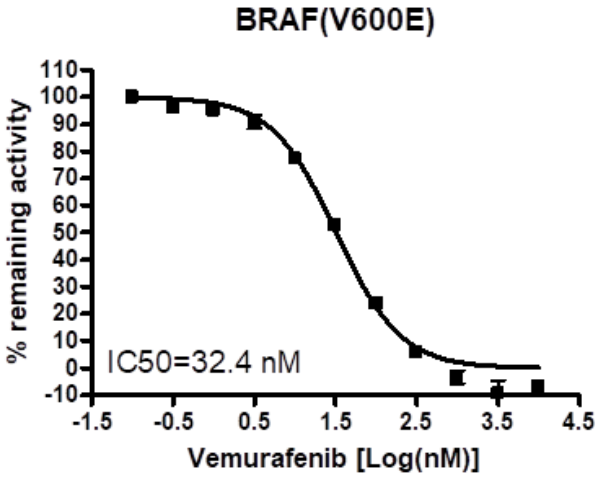
7) Initiate reaction by adding 20 µl of diluted B-Raf(V600E) or B-Raf(WT) enzyme to the wells designated “Positive Control” and "Test Inhibitor Control". Incubate at 30˚C for 45 minutes.

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.

10) Measure luminescence using the microplate reader.

Example of Assay Results:



Inhibition of B-Raf(V600E) enzyme by Vemurafenib, measured using the *B-Raf(V600E) kinase assay kit* (Cat. #48688). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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

RELATED PRODUCTS:

Product Name	Catalog #	Size
BRAF, GST-tag	40065	10 µg
BRAF (V600E), GST-tag	40533	10 µg
BRAF/p50, FLAG-tag	40005	10 µg
aRAF, His-tag	40005	10 µg
cRAF (RAF1)	40008	10 µg
Sorafenib Tosylate	27014	100 mg
Chidamide	27202	1 mg