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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.

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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*

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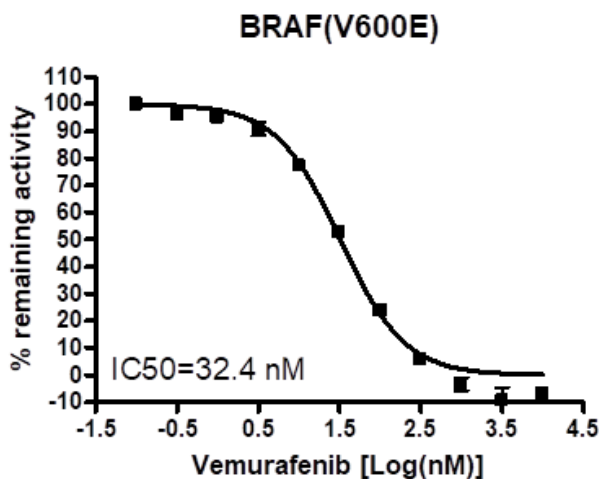
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freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

- 7) Initiate reaction by adding 20 μ l 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 μ l 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*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
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

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