Description

BRAF is a serine-threonine kinase activated by the RAS GTPases in response to growth factor stimulation or oncogenic insult. Upon stimulation, BRAF phosphorylates the kinase MEK1 (dual specificity mitogen-activated protein kinase kinase 1), resulting in the activation of the MAP-kinase (mitogen-activated protein) cascade. The MAP-kinase signaling pathway controls cell growth, and dysregulation of this pathway is frequently observed in cancer. Inherited BRAF mutations cause birth defects, while cancer-associated mutations are frequently observed in melanoma. BRAF is a validated therapeutic target for anti-cancer drugs, notably in melanoma. The BRAF (WT) Kinase Assay Kit is designed to measure BRAF(WT) kinase activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The BRAF (WT) Kinase Assay Kit comes in a convenient 96-well format, with enough purified recombinant BRAF enzyme, BRAF substrate, ATP and Kinase Buffer 1 for 100 enzyme reactions.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40065	BRAF(WT)*	5 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 concentration of BRAF wild-type (WT) is lot-specific and will be indicated on the tube containing the enzyme**

Materials Required but Not Supplied

Name	Catalog #
Kinase-Glo MAX	Promega, #V6071
Dithiothreitol (DTT, 1 M; 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 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 of 5x Kinase Buffer 1)

2. Prepare the Master Mix (25 µl per well): N wells x (6 µl of 5x Kinase Buffer 1 + 1 µl of ATP (500 µM) + 10 µl of 5X Raf substrate + 8 µl of water). Add 25 µl to every well.

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 (Diluent Solution). The diluent solution contains buffer and the same concentration of solvent (for example DMSO) as the inhibitor solution.

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 BRAF (WT) enzyme on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Dilute BRAF(WT) to 2.5 ng/µl with 1x Kinase Buffer 1.

   Note: BRAF enzymes are sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Although we do not recommend it, if not using all the wells of the assay at once calculate the amount required for the assay, dilute only the amount sufficient for the assay and aliquot the remaining undiluted **BRAF** enzyme. Store single use aliquots at -80°C. Do not re-use thawed aliquots or diluted enzyme.

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

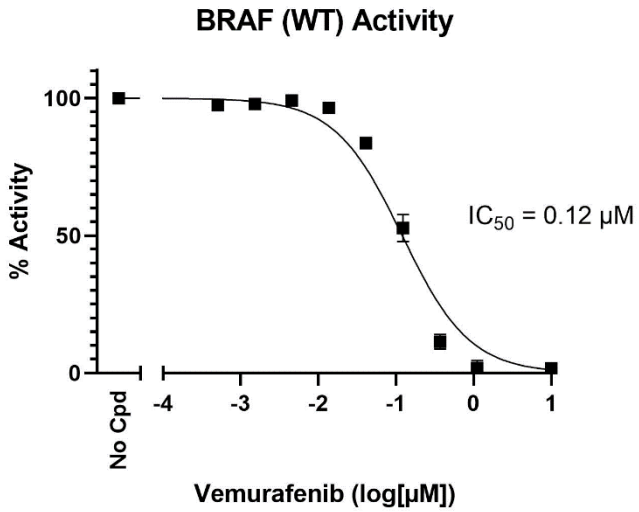
Component	Blank	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor			5 µl
Diluent Solution (No inhibitor)	5 µl	5 µl	
1x Kinase buffer	20 µl		
BRAF (WT) (2.5 ng/µl)		20 µl	20 µl
Total	50 µl	50 µl	50 µl

8. Thaw Kinase-Glo Max reagent.

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

10. Measure luminescence using a microplate reader.

Example Results



Inhibition of BRAF(WT) enzyme by Vemurafenib, measured using the BRAF(WT) Kinase Assay Kit (BPS Bioscience #78316). Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
BRAF, GST-tag	40065	10 µg
BRAF (V600E), GST-tag	40533	10 µg
BRAF (V600E) Kinase Assay Kit	48688	96 reactions
BRAF/p50, FLAG-tag	40005	10 µg
aRAF, His-tag	40010	10 µg
cRAF (RAF1)	40008	10 µg
Sorafenib Tosylate	27014	100 mg
Chidamide	27202	1 mg