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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
B08-13CG -05	5 µg
B08-13CG -10	10 µg

BRAF (R506_K507insVLR), Active

Recombinant human protein expressed in Sf9 cells

Catalog # B08-13CG
Lot # K1563-2

Product Description

Recombinant human BRAF (R506_K507insVLR) (381-end or exon 9-18) protein was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_004333.

Gene Aliases

BRAF (Ex9-18), BRAF1, RAFB1, B-raf, B-raf 1, MGC126806, MGC138284

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

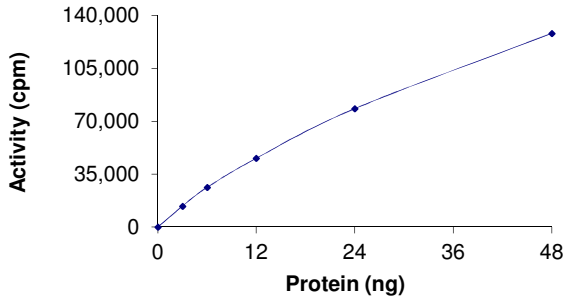
Scientific Background

BRAF is a member of the RAF family that is activated by members of the Ras family upon growth factor-induced stimulation. Active Ras can induce heterodimerization of cRaf and BRAF and this may explain the observed cooperativity of cRaf and BRAF in cells responding to growth factor signals (1). Activating mutations in the BRAF gene are present in a large percentage of human malignant melanomas and in a proportion of colon cancers. The vast majority of these mutations result in a valine to glutamic acid change at residue 599 within the activation segment of BRAF (2).

References

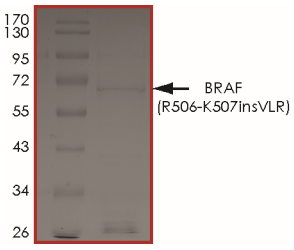
1. Weber, C K. et al: Active Ras induces heterodimerization of cRaf and BRaf. Cancer Res. 2001 May 1;61(9):3595-8.
2. Mercer, K E. et al: Raf proteins and cancer: B-Raf is identified as a mutational target. Biochim Biophys Acta. 2003 Jun 5;1653(1):25-40.

Specific Activity



The specific activity of BRAF (R506_K507insVLR) was determined to be **220 nmol/min/mg** in a coupled assay as per activity assay protocol.

Purity



The purity of BRAF (R506_K507 insVLR) protein was determined to be **>70%** by densitometry, approx. MW **69 kDa**.

BRAF (R506_K507insVLR), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	B08-13CG
Specific Activity	220 nmol/min/mg
Lot #	K1563-2
Purity	>70%
Concentration	0.05 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: B08-13CG)

Active BRAF (R506_K507insVLR) (0.05µg/µl) diluted with Kinase Dilution Buffer III (Catalog #: K23-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active BRAF for optimal results).

Kinase Dilution Buffer VII (Catalog #: K23-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA.

Kinase Assay Buffer I (Catalog #: K01-09)

Buffer components: 25mM MOPS pH 7.2, 12.5mM β-glycerol-phosphate, 25mM $MgC1_2$, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[^{33}P]-ATP Assay Cocktail

Prepare 250µM [^{33}P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [^{33}P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 1ml aliquots at –20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer I (Catalog #: K01-09). Store 200µl aliquots at –20°C.

Substrate (Catalog #: M02-14BG)

Unactive MEK1 (Catalog #: M02-14BG) and ERK1 (Catalog #: M29-14G) were activated in a coupled reaction. Myelin Basic Protein (MBP) (Catalog #: M42-51N) diluted in distilled H_2O to a final concentration of 1mg/ml was subsequently used as a substrate for the activated ERK1.

Assay Protocol

Step 1. Thaw [^{33}P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.

Step 2. Thaw the Active BRAF (R506_K507insVLR), Kinase Assay Buffer, Unactive ERK1 and Unactive MEK1 on ice. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:

- **Component 1.** 10µl of diluted Active BRAF (R506_K507insVLR) (Catalog #B08-13CG)
- **Component 2.** 0.25µl of Unactive MEK1 (0.2µg/µl) (Catalog #M02-14BG)
- **Component 3.** 0.25µl of Unactive ERK1 (0.2µg/µl) (Catalog #M29-14G)
- **Component 4.** 4.5µl of Kinase Dilution Buffer (Catalog #K23-09)

Step 3. Start the reaction by the addition of 5 µl [^{33}P]-ATP Assay Cocktail solution and incubate in a water bath at 30°C for 15 minutes.

Step 4. After the 15 minute incubation period, add 5µl of MBP substrate on ice (1 mg/ml) (Catalog #M42-51N) bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.

Step 5. Set up the blank control as outlined in step 4, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20 µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H_2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [^{33}P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [^{33}P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of ^{33}P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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