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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
B08-19FG -05	5 µg
B08-19FG -10	10 µg

SRGAP3-BRAF (Sex12Bex9), Active

Recombinant human protein expressed in Sf9 cells

Catalog # B08-19FG

Lot # K1685-3

Product Description

Recombinant human fusion SRGAP3 (1-513 exon12)-BRAF (381-end or exon9-18) protein was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number of SRGAP3 is NM_014850 and BRAF is NM_004333.

Gene Aliases

SRGAP3: ARHGAP14; MEGAP; SRGAP2; WRP
BRAF: BRAF1, RAFB1, B-raf, 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.

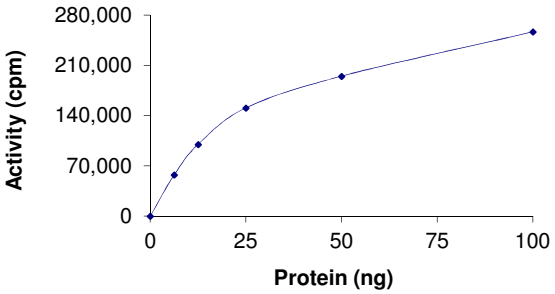
Scientific Background

KIAA1549-BRAF is a gene fusion resulting from a tandem duplication event involving the BRAF kinase gene that have recently been identified as the most frequent genetic alteration in many cancers. The KIAA1549-BRAF fusion typically results from a 2.0 Mb tandem duplication in chromosome band 7q34 (1). The KIAA1549:BRAF fusion gene is considered a driver genetic event in pilocytic astrocytoma and many other pediatric brain neoplasms. KIAA1549-BRAF fusion gene and BRAF (V600E) mutation may be responsible for deregulation of the Ras-RAF-ERK signaling pathway in many brain cancers (2).

References

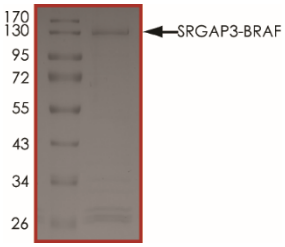
1. Dougherty, M J. et al: Activating mutations in BRAF characterize a spectrum of pediatric low-grade gliomas. Neuro Oncol. 2010 Jul;12(7):621-30.
2. Badiali, M. et al: KIAA1549-BRAF fusions and IDH mutations can coexist in diffuse gliomas of adults. Brain Pathol. 2012 Nov;22(6):841-7.

Specific Activity



The specific activity of SRGAP3-BRAF (Sex12Bex9) was determined to be **250 nmol/min/mg** in a coupled assay as per activity assay protocol.

Purity



The purity of SRGAP3-BRAF (Sex12Bex9) protein was determined to be **>80%** by densitometry, approx. MW **128 kDa**.

SRGAP3-BRAF (Sex12Bex9), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	B08-19FG
Specific Activity	250 nmol/min/mg
Lot #	K1685-3
Purity	>80%
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 #: B08-19FG)

Active SRGAP3-BRAF (Sex12Bex9) (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 SRGAP3-BRAF (Sex12Bex9), 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 SRGAP3-BRAF (Sex12Bex9) (Catalog #B08-19FG)
- **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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