

KIAA1549-BRAF (Kex16Bex9), Active

Recombinant human protein expressed in Sf9 cells

Catalog # B08-198G

Lot # K1563-1

Product Description

Recombinant human fusion KIAA1549 (1320-1749 exon16)-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 KIAA1549 is [NM_001164665](#) and BRAF is [NM_004333](#).

Gene Aliases

KIAA1549: none

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.

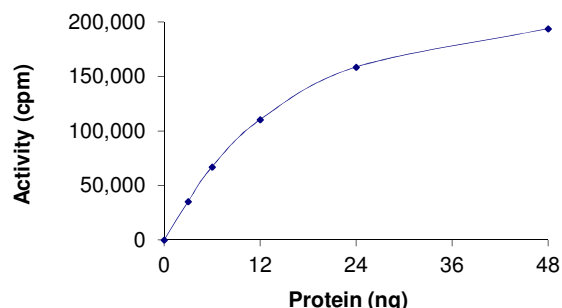
Scientific Background

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

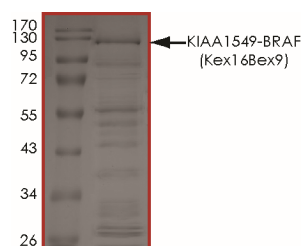
- Dougherty, M. J. et al: Activating mutations in BRAF characterize a spectrum of pediatric low-grade gliomas. *Neuro Oncol*. 2010 Jul;12(7):621-30.
- Badiali, M. et al: KIAA1549-BRAF fusions and IDH mutations can coexist in diffuse gliomas of adults. *Brain Pathol*. 2012 Nov;22(6):841-7.

Specific Activity



The specific activity of KIAA1549-BRAF (Kex16Bex9) was determined to be **550 nmol/min/mg** in a coupled assay as per activity assay protocol.

Purity



The purity of KIAA1549-BRAF (Kex16Bex9) protein was determined to be **>70%** by densitometry, approx. MW **125 kDa**.

KIAA1549-BRAF (Kex16Bex9), Active

Recombinant human protein expressed in Sf9 cells

Catalog # B08-198G

Specific Activity 550 nmol/min/mg

Lot # K1563-1

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 #: B08-19BG)

Active KIAA1549-BRAF (Kex16Bex9) (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 MgCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-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 H₂O to a final concentration of 1mg/ml was subsequently used as a substrate for the activated ERK1.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active KIAA1549-BRAF (Kex16Bex9), 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 KIAA1549-BRAF (Kex16Bex9) (Catalog #B08-19BG)
 - 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 [³³P]-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 H₂O.
- 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 H₂O) 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-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 ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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