

FAM131B-BRAF (Fex2Bex9), Active

Recombinant human protein expressed in Sf9 cells

Catalog # B08-19EG Lot # K1743-1

Product Description

Recombinant human fusion FAB131B (1-30 exon2)-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 <u>BC050543</u> and BRAF is <u>NM 004333</u>.

Gene Aliases

FAM131B: (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

- 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.
- Badiali, M. et al: KIAA1549-BRAF fusions and IDH mutations can coexist in diffuse gliomas of adults. <u>Brain Pathol.</u> 2012 Nov;22(6):841-7.

Specific Activity



The specific activity of FAM131B-BRAF (Fex2Bex9) was determined to be **2,100 nmol/min/mg** in a coupled assay as per activity assay protocol.

Catalog #

B08-19EG -05

B08-19EG -10

Aliquot Size

5 µg

10 µg

Purity



The purity of FAM131B-BRAF (Fex2Bex9) was determined to be >70% by densitometry, approx. MW 75 kDa.

FAM131B-BRAF (Fex2Bex9), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	B08-19EG
Specific Activity	2,100 nmol/min/mg
Lot #	K1743-1
Purity	>70%
Concentration	0.1 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 #: B08-19EG)

Active FAM131B-BRAF (Fex2Bex9) $(0.1\mu g/\mu l)$ 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.

[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 200 μ l 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 FAM131B-BRAF (Fex2Bex9), 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 FAM131B-BRAF (Fex2Bex9) (Catalog #B08-19EG)
 - Component 2. 0.25µl of Unactive MEK1 (0.2µg/µl) (Catalog #M02-14BG)
 - **Component 3.** 0.25 μ l of Unactive ERK1 (0.2 μ g/ μ l) (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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