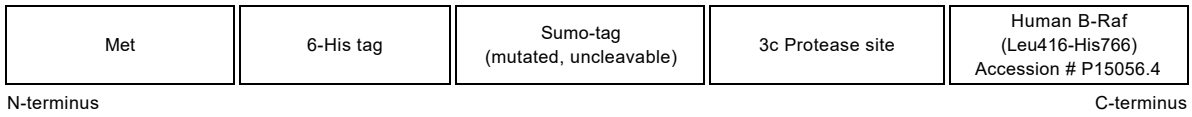
Recombinant Human B-Raf kinase domain His-tag

Catalog Number: 11831-BR

DESCRIPTION

Source

Spodoptera frugiperda, *Sf* 21 (baculovirus)-derived human B-Raf protein



N-terminal Sequence Analysis	Protein identity confirmed by mass spectrometry
Predicted Molecular Mass	53 kDa

SPECIFICATIONS

SDS-PAGE	49-58 & 27-33 kDa, under reducing conditions
Activity	Measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a synthetic peptide substrate. The specific activity is >20 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>85%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mg/mL BSA, pH 7.5
- Recombinant Human B-Raf kinase domain His-tag Protein (rhB-Raf) (Catalog # 11831-BR)
- Poly (4:1 Glu, Tyr), 1 mg/mL stock in 25 mM Tris, pH 7.5
- Adenosine triphosphate (ATP), 10 mM stock in deionized water
- ADP-Glo™ Kinase Assay Kit (Promega)
- White 96-well Plate
- Plate Reader with Luminescence Read Capability

Assay

1. Dilute rhB-Raf to 20 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 200 µM ATP and 25 µg/mL Poly (4:1 Glu, Tyr) in Assay Buffer.
3. Combine equal volumes of 20 µg/mL rhB-Raf and Substrate Mixture. Create a Substrate Control containing equal volumes of Assay Buffer and Substrate Mixture.
4. Incubate at room temperature for 40 minutes in the dark.
5. After incubation, transfer 10 µL of each reaction to wells of a white plate.
6. Terminate the reaction and deplete the remaining ATP by adding 10 µL of ADP-Glo™ Reagent (supplied in kit) to all wells.
7. Incubate at room temperature for 40 minutes in the dark.
8. Add 20 µL Kinase Detection Reagent (supplied in kit) to all wells.
9. Incubate at room temperature for 30 minutes in the dark.
10. Read plate in Luminescence endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Luminescence* (RLU) x Conversion Factor** (pmol/RLU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Control

**Derived from ADP-Glo™ Kinase Assay Kit protocol (Promega)

Final Assay Conditions

Per Reaction:

- rhB-Raf: 10 µg/mL
- ATP: 100 µM
- Poly (4:1 Glu, Tyr): 12.5 µg/mL

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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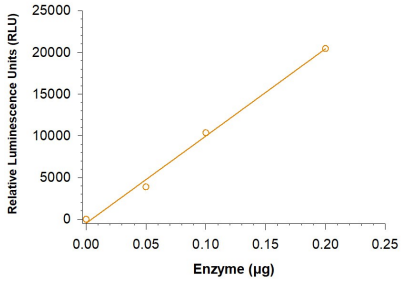
DATA

Enzyme Activity



Recombinant Human B-Raf kinase domain His-tag Enzyme Activity. Recombinant Human B-Raf kinase domain His-tag (Catalog # 11831-BR) is measured by its ability to transfer phosphate from adenosine triphosphate (ATP) to a synthetic peptide substrate.

BACKGROUND

Serine/Threonine-protein kinase B-Raf (B-Raf), also referred to as proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B is a broadly expressed cytoplasmic kinase from the TKL (Tyrosine Kinase-Like) group of Serine/Threonine protein kinase family that functions as a critical gatekeeper in the MAP kinase/ERKs signaling pathway that controls cell division, proliferation and survival (1,2). B-Raf is one of three highly conserved RAF kinase isoforms that are composed of three conserved domains characteristic of the subfamily (3). Conserved region 1 (CR1) contains a Ras-GTP-binding self-regulatory domain that autoinhibits B-Raf's kinase domain thus regulating B-Raf signaling (3,4). Conserved region 2 (CR2) is a serine-rich hinge region, and conserved region 3 (CR3), is a conserved bi-lobal catalytic protein kinase domain where the smaller lobe contains the P-loop responsible for ATP binding and the larger lobe contains the activation loop that binds substrate proteins with the active site located in the cleft between the two lobes (5). B-Raf must bind Ras-GTP which causes a conformational change to its active dimeric conformation and phosphorylation (6,7). Although similar in structure, B-Raf is the Raf family member that has been shown to function as the main and most potent activator of the MAPK cascade via targeted MEK phosphorylation (3,8). Inherited mutations in the BRAF gene can cause Cardiofaciocutaneous, Noonan, and Leopard syndromes (9). More than 30 mutations of the BRAF gene, including the V600E mutation within the activation segment that causes constitutive signaling, have been associated with diverse human cancer types including colorectal cancer, melanoma, papillary thyroid carcinoma, non-small-cell lung carcinoma, glioblastoma (10-14), as well as inflammatory diseases such as Erdheim–Chester disease (1,2,15). As BRAF is a well-understood target (16) and inhibition via approved drugs targeting B-Raf specifically and in combination have shown improved survival and response rate for several mutation-related cancers including solid tumors (1,17,18,19), there is significant ongoing research in development of additional pharmacological inhibitors for B-Raf kinase.

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