B-Raf (V600E) [GST-tagged]

Kinase

Alternate Names: Serine/threonine-protein kinase B-raf, BRAF, B-RAF1, BRAF1, RAFB1

Cat. No. **66-0023-050**
Lot. No. **30302**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. B-Raf is known as v-raf murine sarcoma viral homolog B1 which is a proto-oncogene. It is a member of the Raf (Rapidly accelerated fibrosarcoma) kinase family of proteins. There are three Raf kinase family members, all serine/threonine kinases, identified as: A-Raf, B-Raf and C-Raf (Rahman *et al.*, 2013). Cloning of the B-Raf gene was first described by Sithanandam *et al.* (1990). Members of the Raf family are involved in a variety of cellular activities, including growth, survival, differentiation, and transformation. An oncogene encodes B-Raf and constitutively active mutations of B-Raf are widely known to correlate with human cancer development. B-Raf is the most effective RAF kinase in terms of induction of MEK/ERK activity. However, the mechanisms involved in B-Raf regulation remain unclear. Recent studies have shown that B-Raf is involved in the ubiquitin-proteasome

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Physical Characteristics

Species: human

Source: baculovirus expression vector system

Quantity: 50 µg

Concentration: 0.73 mg/ml

Formulation: 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% ß-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

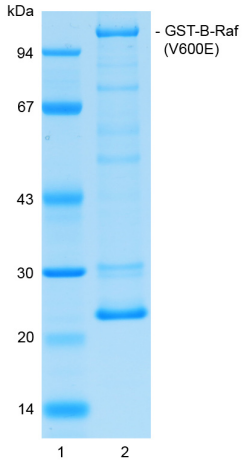
Molecular Weight: ~111.9 kDa
Purity: >50% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg GST-B-Raf (V600E)



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of GST-B-Raf (V600E) was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. Initially, GST-B-Raf (V600E) (diluted in 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 1 mg/ml BSA, 10 mM DTT) was incubated with MKK1 (0.4 µg), p42MAPK (1.4 µg) and ATP (0.1 mM) in 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 10 mM MgAc, 10 mM DTT buffer for 30 minutes at 30°C. A sample of this GST-B-Raf (V600E) reaction was then incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of MBP substrate (0.33 mg/ml) and [γ-32P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate. The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

GST-B-Raf (V600E) specific activity:
51723 Units/mg (37758 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: Myelin Basic Protein (MBP)

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

pathway. RNF149 (RING finger protein 149), a known ubiquitin E3 ligase, interacts with wild-type B-Raf - not mutant B-Raf - inducing ubiquitylation, followed by proteasome-dependent degradation of B-Raf (Hong *et al.*, 2012). It was also recently discovered that B-Raf can be modified by Lys63-linked polyubiquitylation at lysine 578 within its kinase domain after activation by gain of a constitutively active mutation or by epidermal growth factor (EGF) stimulation. However, further studies are needed to identify the specific E3 ligase(s) and deubiquitylating enzyme(s) responsible for the positive and negative regulation of B-Raf Lys63-linked polyubiquitylation (An *et al.*, 2013).

References:

An L, Jia W, Yu Y, Zou N, Liang L, Zhao Y, *et al.* (2013) Lys63-linked polyubiquitination of BRAF at lysine 578 is required for BRAF-mediated signaling. *Sci Rep* **3**, 2344.

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Hong SW, Jin DH, Shin JS, Moon JH, Na YS, Jung KA, *et al.* (2012) Ring finger protein 149 is an E3 ubiquitin ligase active on wild-type v-Raf murine sarcoma viral oncogene homolog B1 (BRAF). *J Biol Chem* **287**, 24017-24025.

Rahman MA, Salajegheh A, Smith RA and Lam AK (2013) B-Raf mutation: a key player in molecular biology of cancer. *Exp Mol Pathol* **95**, 336-342.

Sithanandam G, Kolch W, Duh FM and Rapp UR (1990) Complete coding sequence of a human B-raf cDNA and detection of B-raf protein kinase with isozyme specific antibodies. *Oncogene* **5**, 775-1780.

Ziai J and Hui P (2012) BRAF mutation testing in clinical practice. *Expert Rev Mol Diagn* **12**, 127-38.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCP
KERAEISMLEGAVLDIRYGVSRIAYSKD
FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGD
HVTHPDFMLYDALDVVLYMDPMCLDAFP
KLVCFKKRIEAIPQIDKYLKSSKYIAWPLQG
WQATFGGGDHPPKSDLEVLFQGPLGSPN
SRVD***A***ALSGGGGGGAEPGQALFNGDME
PEAGAGAGAAASSAADPAIPEEVWNIKQ
MIKLTQEHIEALLDKFGGEHNPPSIYLEAY
EEYTSKLDALQQREQQLLESLGNGTD
FSVSSSASMDTVTSSSSSSLSVLPSSLS
VFQNPTDVARSNPKSPQKPIVRVFLPNKQRT
VVPARCGVTVRDSLKKALMMRGLIPECCA
VYRIQDGEKKPIGWDTDISWLTGEELHVEV
LENVPLTTHNFVRKTFFTLAFCDFCRKLLFQG
FRCQTCGYKFHQRCSTEVPLMCVNYDQLDLL
FVSKFFEHHPIPQEEASLAETALTSGSSPSA
PASDSIGPQILTSPSPSKSIPIPQPFRPAD
EDHRNQFGQRDRSSSAPNVHINTIEPVNID
DLIRDQGFRGDGGSTTGLSATPPASLPGSLT
NVKALQKSPGPQRERKSSSSSEDRNRMKTL
GRRDSSDDWEIPDGQITVGQRIGSGSFGTVYK
GKWHGDVAVKMLNVTAPTPQQLQAFKNEVGVL
RKTRHVNILLFMGYSTKPQLAIVTQWCEGSS
LYHHLHIIETKFEMIKLIDIARQTAQGMDYL
HAKSIIHRDLKSNNIFLHEDLTVKIGDFGLAT
EKSRWSGSHQFEQLSGSILWMAPEVIRMQD
KNPYSFQSDVYAFGIVLYELMTGQLPYSNIN
NRDQIIFMVGRGYLSPDLSKVRSNCPKAMKRL
MAECLKKKRDERPLFPQILASIELLARSLP
KIHRSASEPSLNRAGFQTEDFSLYACASPKT
PIQAGGYGAFPVH

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
B-Raf (regular text): Start ***bold italics*** (amino acid residues 2-766).
The enzyme has a V600E mutation to mimic the enzyme activating mutation found in a high number of malignant melanomas and other cancers (Ziai and Hui, 2012).
Accession number: NP_004324.2

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