

## ProQinase™ B-RAF V600E

B-Raf proto-oncogene, serine/threonine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: BRAF

Synonyms: BRAF1, RAFB1, B-raf 1, NS7

Product No.: 0404-0000-1

Lot: 002

**Description:** Human B-RAF, C-terminal fragment, amino acids Q<sub>417</sub>-H<sub>766</sub> (as in [NCBI/Protein](#) entry NP\_004324.2), V600E mutation, N-terminal GST-HIS6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** B-RAF V600E, Lot 002, was confirmed as B-RAF VE by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>Fusion Protein</sub>:** 69,157 Da

**Expression host:** Sf9 insect cells

**Purification:** GST-Affinity Chromatography

**Activation:** This kinase was not activated by special procedures

**Storage buffer:** 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

**Storage temperature:** -80°C

For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

**Protein concentration:** 0.190 µg/µl  
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

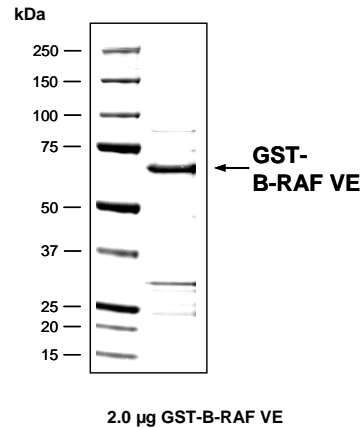
**Biochemical Parameters:**

Specific kinase activity (P<sub>i</sub> transfer): 197 pmol/µg × min  
ATP-K<sub>M</sub>: 3.6 µM

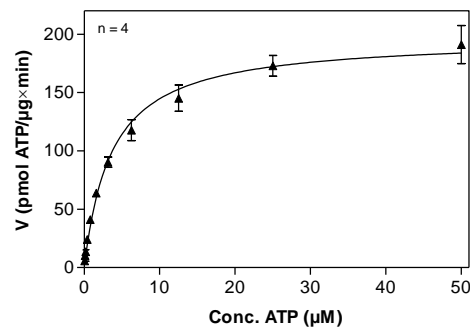
**Additional assay technology:**

B-RAF V600E Lot 002 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

**B-RAF V600E Lot 002:  
Coomassie stain**



**B-RAF V600E Lot 002:  
Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP**



**Determination of K<sub>M</sub> value & Specific activity:**

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg/ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: MEK1-KM, 60 µg/ml
  - Kinase: 0.5 µg/ml
- Filter binding assay
  - MSFC membrane (Millipore)

## ProQinase™ B-RAF V600E

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GST-B-RAF V600E Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMG <b>HHHHHG</b>	RRRASVAAGI	240
241	<b>LVPRGSPGLD</b>	GICSRNS <b>QKS</b>	<b>PGPQREKSS</b>	<b>SSSEDRNRMK</b>	<b>TLGRRDSSDD</b>	<b>WEIPDGQITV</b>	300
301	<b>GQRIGSGSFG</b>	<b>TVYKQKWHGD</b>	<b>VAVKMLNVTA</b>	<b>PTPQQLQAFK</b>	<b>NEVGVLKTR</b>	<b>HVNILLFMGY</b>	360
361	<b>STKPQLAIVT</b>	<b>QWCEGSSLYH</b>	<b>HLHIETKFE</b>	<b>MIKLIDIARQ</b>	<b>TAQGMDYLHA</b>	<b>KSIHRDLKS</b>	420
421	<b>NNIFLHEDLT</b>	<b>VKIGDFGLAT</b>	<b>EKSRWSGSHQ</b>	<b>FEQLSGSILW</b>	<b>MAPEVIRMQD</b>	<b>KNPYSFQSDV</b>	480
481	<b>YAFGIVLYEL</b>	<b>MTGQLPYSNI</b>	<b>NNRDQIIFMV</b>	<b>GRGYLSPDLS</b>	<b>KVRSNCPKAM</b>	<b>KRLMAECLKK</b>	540
541	<b>KRDERPLFPQ</b>	<b>ILASIELLAR</b>	<b>SLPKIHRAS</b>	<b>EPSLNRAGFQ</b>	<b>TEDFSLYACA</b>	<b>SPKTPIQAGG</b>	600
601	<b>YGAFPVH</b>						660

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:** B-RAF fragment **boxed:** V600E

B-RAF wt <sup>1</sup> Amino Acid Sequence							
1	MAALSGGGGG	GAEPGQALFN	GDMEPEAGAG	AGAAASSAAD	PAIPEEVWNI	KQMIKLTQEH	60
61	IEALLDKFGG	EHNPPSIYLE	AYEEYTSKLD	ALQOREQQLL	ESLGNGTDFS	VSSSASMDTV	120
121	TSSSSSSLSV	LPSSLSVFQN	PTDVARSNPK	SPQKPIVRVF	LPNKQRTVVP	ARCGVTVRDS	180
181	LKKALMMRGL	IPECCAVYRI	QDGEKKPIGW	DTDISWLTGE	ELHVEVLENV	PLTTHNFVRK	240
241	TFFTLAFCDF	CRKLLFQGFR	CQTCGYKFHQ	RCSTEVPLMC	VNYDQLDLLF	VSKFFEHHPI	300
301	PQEEASLAET	ALTSGSSPSA	PASDSIGPQI	LTSPSPSKSI	PIPQPPRPAD	EDHRNQFGQR	360
361	DRSSAPNVH	INTIEPVNID	DLIRDQGFRG	DGGSTTGLSA	TPPASLPGSL	TNVKAL <b>QKSP</b>	420
421	<b>GPQREKSSS</b>	<b>SSEDRNRMKT</b>	<b>LGRRDSSDDW</b>	<b>EIPDGQITVG</b>	<b>QRIGSGSFGT</b>	<b>VYKQKWHGDV</b>	480
481	<b>AVKMLNVTAP</b>	<b>TPQQLQAFKN</b>	<b>EVGVLKTRH</b>	<b>VNILLFMGYS</b>	<b>TKPQLAIVTQ</b>	<b>WCEGSSLYHH</b>	540
541	<b>LHIETKFEM</b>	<b>IKLIDIARQT</b>	<b>AQGMDYLHAK</b>	<b>SIIHRDLKSN</b>	<b>NIFLHEDLTV</b>	<b>KIGDFGLATV</b>	600
601	<b>KSRWSGSHQF</b>	<b>EQLSGSILWM</b>	<b>APEVIRMQDK</b>	<b>NPYSFQSDVY</b>	<b>AFGIVLYELM</b>	<b>TGQLPYSNIN</b>	660
661	<b>NRDQIIFMVG</b>	<b>RGYLSPLSK</b>	<b>VRSNCPKAMK</b>	<b>RLMAECLKKK</b>	<b>RDERPLFPQI</b>	<b>LASIELLARS</b>	720
721	<b>LPKIHRSASE</b>	<b>PSLNRAGFQT</b>	<b>EDFSLYACAS</b>	<b>PKTPIQAGGY</b>	<b>GAFPVH</b>		780

**blue:** B-RAF sequence expressed in recombinant protein **Red:** variant in recombinant protein

<sup>1</sup>[NCBI/Protein](#) accession number NP\_004324.2