

## 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)

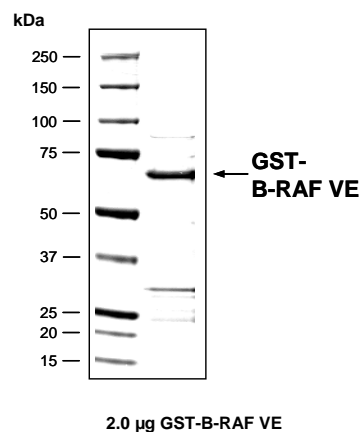
### Biochemical Parameters:

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

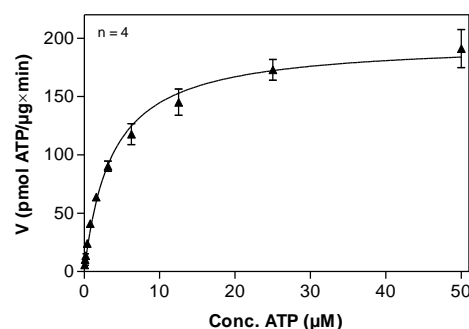
### Additional assay technology:

B-RAF V600E Lot 002 was also successfully tested by ProQinase 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)

## B-RAF V600E

Product No.: 0404-0000-1

GST-B-RAF V600E Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	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	PMGHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSRNSQKS	PGPQERKSS	SSSEDNRMK	TLGRRDSSDD	WEIPDGQITV	300
301	GQRIGSGSFG	TVYKKGWHGD	VAVKMLNVTA	PTPQQLQAFK	NEVGVLKTR	HVNILLFMGY	360
361	STKPQLAIVT	QWCEGSSLYH	HLHIETKFE	MIKLIDIARQ	TAQGM DYLHA	KSIHRDLKS	420
421	NNIFLHEDLT	VKIGDFGLAT	EKSRWSGSHQ	FEQLSGSILW	MAPEVIRMQD	KNPYSFQSDV	480
481	YAFGIVLYEL	MTGQLPYSNI	NNRDQIIFMV	GRGYLSPDLS	KVRSNCPKAM	KRLMAECLKK	540
541	KRDERPLFPQ	ILASIELLAR	SLPKIHRAS	EPSLNRAGFQ	TEDFSLYACA	SPKTPIQAGG	600
601	YGAFPVH						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	VSSASAMDTV	120
121	TSSSSSSLSV	LPSSLSVFQN	PTDVARSNPK	SPQKPIRVVF	LPNKQRTVVP	ARCGVTVRDS	180
181	LKKALMMRGL	IPECCAVYRI	QDGEKKPIGW	DTDISWLTGE	ELHVEVLENV	PLTTHNFVRK	240
241	TFFTLAFCDF	CRKLLFQGF	CQTCGYKFHQ	RCSTEVPLMC	VNYDQLDLLE	VSKFFEHHP	300
301	PQEEASLAET	ALTSGSSPSA	PASDSIGPQI	LTSPSPSKSI	PIPQPPRPAD	EDHRNQFGQR	360
361	DRSSAPNVH	INTIEPVNID	DLIRDQGFRG	DGGSTTGLSA	TPPASLPGSL	TNVKALQKSP	420
421	GPQERKSSS	SSEDNRMK	LGRRDSSDDW	EIPDGQITVG	QRIGSGSFGT	VYKKGWHGDV	480
481	AVKMLNVTAP	TPQQLQAFKN	EVGVLKTRH	VNILLFMGYS	TKPQLAIVTQ	WCEGSSLYHH	540
541	LHIETKFEM	IKLIDIARQT	AQGM DYLHAK	SIIHRDLKSN	NIFLHEDLTV	KIGDFGLATV	600
601	KSRWSGSHQF	EQLSGSILWM	APEVIRMQDK	NPYSFQSDVY	AFGIVLYELM	TGQLPYSNIN	660
661	NRDQIIFMVG	RGYLSPLSK	VRSNCPKAMK	RLMAECLKKK	RDERPLFPQI	LASIELLARS	720
721	LPKIHRASE	PSLNRAGFQT	EDFSLYACAS	PKTPIQAGGY	GAFPVH		780

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

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