

## B-RAF wt

B-Raf proto-oncogene, serine/threonine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: BRAF

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

Product No.: 0403-0000-1

Lot: 001

**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), N-terminal GST-HIS<sub>6</sub> fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

**Product identity:** B-RAF wt Lot 001, was confirmed as B-RAF by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW**<sub>Fusion Protein</sub>: 69,127 Da

**Expression host:** Sf9 insect cells

**Purification:** GST-Affinity Chromatography

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

**Storage buffer:** 50 mM TRIS-HCl pH 7.5, 100 mM NaCl, 5 mM DTT, 15 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:** 0.294 µg/µl

(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

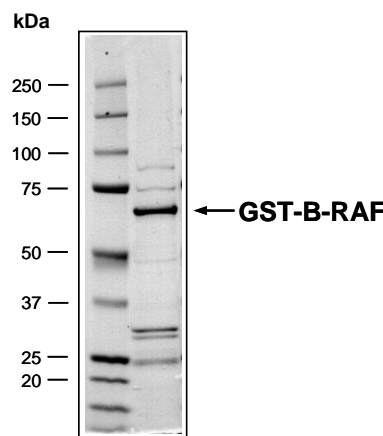
Specific kinase activity (P<sub>i</sub> transfer): 139 pmol/µg × min

ATP-K<sub>M</sub>: 2.1 µM

### Additional assay technology:

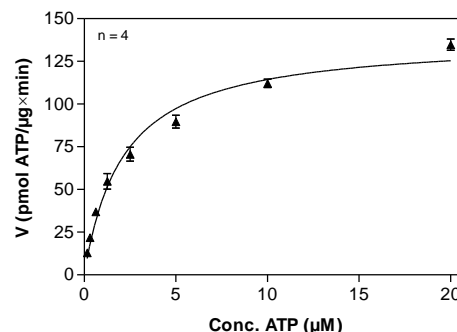
B-RAF wt Lot 001 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

### B-RAF wt Lot 001: Coomassie stain



2.0 µg GST-B-RAF

### B-RAF wt Lot 001: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



#### • 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 K97M, 40 µg/ml
- Kinase: 0.5 µg/ml

- Filter binding assay
- MSFC membrane (Millipore)

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GST-B-RAF wt 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	PMG <b>HHHHHG</b>	RRRASVAAGI	240
241	<b>LVPRGSPGLD</b>	GICSRNS <b>QKS</b>	<b>PGPQERKSS</b>	<b>SSSEDRNRMK</b>	<b>TLGRRDSSDD</b>	<b>WEIPDGQITV</b>	300
301	<b>GQRIGSGSFG</b>	<b>TVYKKGWHGD</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>TAQGM DYLHA</b>	<b>KSIHRDLKS</b>	420
421	<b>NNIFLHEDLT</b>	<b>VKIGDFGLAT</b>	<b>VKSRWSGSHQ</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 wt fragment **boxed**: variation from RefSeq

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	SPQKPIVRVF	LPNKQRTVVP	ARCGVTVRDS	180
181	LKKALMMRGL	IPECCAVYRI	QDGEKKPIGW	DTDISWLTGE	ELHVEVLENV	PLTTHNFVRK	240
241	TFFTLAFCDF	CRKLLFQGF	CQTCGYKFHQ	RCSTEVPLMC	VNYDQLDLLE	VSKFFEHHPI	300
301	PQEEASLAET	ALTSGSSPSA	PASDSIGPQI	LTSPSPSKSI	PIQPFRPAD	EDHRNQFGQR	360
361	DRSSAPNVH	INTIEPVNID	DLIRDQGFRG	DGGSTTGLSA	TPPASLPGSL	TNVKAL <b>QKSP</b>	420
421	<b>GPQERKSSS</b>	<b>SSEDRNRMKT</b>	<b>LGRRDSSDDW</b>	<b>EIPDGQITVG</b>	<b>QRIGSGSFGT</b>	<b>VYKKGWHGDV</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>AQGM DYLHAK</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>LPKIHRASASE</b>	<b>PSLNRAGFQT</b>	<b>EDFSLYACAS</b>	<b>PKTPIQAGGY</b>	<b>GAFPVH</b>		780

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

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

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