Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ 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_{417}-H_{766} (as in NCBI/Protein entry NP_004324.2), N-terminal GST-HIS_6 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 $MW_{Fusion\ Protein}$: 69,127 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

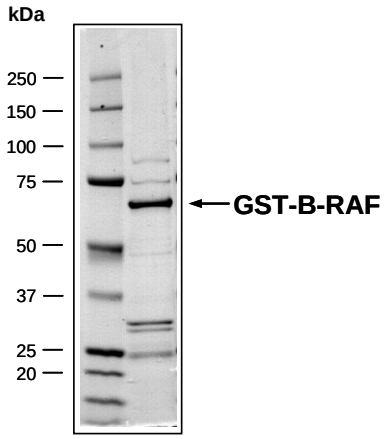
Storage buffer: 50 mM TRIS-HCl pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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)

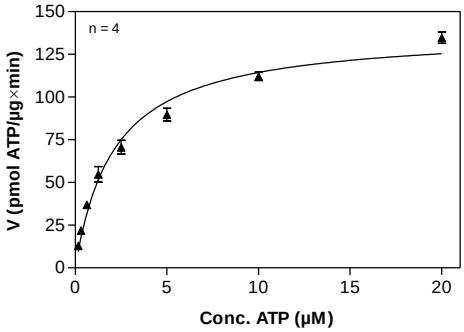
Biochemical Parameters:
Specific kinase activity (P_i transfer): 139 pmol/µg × min
ATP-K_M: 2.1 µM

B-RAF wt Lot 001:
Coomassie stain



2.0 µg GST-B-RAF

B-RAF wt Lot 001:
Determination of V_{max} and K_M value for ATP



- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: MEK1 K97M, 40 µg/ml
  Kinase: 0.5 µg/ml
- Filter binding assay

MSFC membrane (Millipore)

Additional assay technology:
B-RAF wt Lot 001 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

ProQinase™ B-RAF wt

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GST-B-RAF wt Recombinant Fusion Protein Amino Acid Sequence

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1    MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
61   GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI 240
241  LVPRGSPGLD GICSRNSQKS PGPQRERKSS SSSEDRNRMK TLGRRDSSDD WEIPDGQITV 300
301  GQRIGSGSFG TVYKGKWHGD VAVKMLNVTA PTPQQLQAFK NEVGVLRKTR HVNILLFMGY 360
361  STKPQLAIVT QWCEGSSLYH HLHIIETKFE MIKLIDIARQ TAQGMDYLHA KSIIHRDLKS 420
421  NNIFLHEDLT VKIGDFGLAT VKSRWSGSHQ FEQLSGSILW MAPEVIRMQD KNPYSFQSDV 480
481  YAFGIVLYEL MTGQLPYSNI NNRDQIIFMV GRGYLSPDLS KVRSNCPKAM KRLMAECLKK 540
541  KRDERPLFPQ ILASIELLAR SLPKIHRSAS EPSLNRAGFQ TEDFSLYACA SPKTPIQAGG 600
601  YGAFPVH                                                          660
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: B-RAF wt fragment **boxed**: variation from RefSeq

B-RAF wt[1] Amino Acid Sequence

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  1  MAALSGGGGG GAEPGQALFN GDMEPEAGAG AGAAASSAAD PAIPEEVWNI KQMIKLTQEH  60
 61  IEALLDKFGG EHNPPSIYLE AYEEYTSKLD ALQQREQQLL 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 PIPQPFRPAD EDHRNQFGQR 360
361  DRSSSAPNVH INTIEPVNID DLIRDQGFRG DGGSTTGLSA TPPASLPGSL TNVKALQKSP 420
421  GPQRERKSSS SSEDRNRMKT LGRRDSSDDW EIPDGQITVG QRIGSGSFGT VYKGKWHGDV 480
481  AVKMLNVTAP TPQQLQAFKN EVGVLRKTRH VNILLFMGYS TKPQLAIVTQ WCEGSSLYHH 540
541  LHIIETKFEM IKLIDIARQT AQGMDYLHAK SIIHRDLKSN NIFLHEDLTV KIGDFGLATV 600
601  KSRWSGSHQF EQLSGSILWM APEVIRMQDK NPYSFQSDVY AFGIVLYELM TGQLPYSNIN 660
661  NRDQIIFMVG RGYLSPDLSK VRSNCPKAMK RLMAECLKKK RDERPLFPQI LASIELLARS 720
721  LPKIHRSASE PSLNRAGFQT EDFSLYACAS PKTPIQAGGY GAFPVH               780
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blue: B-RAF sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_004324.2

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