Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ BMPR1B

bone morphogenetic protein receptor type 1B

Recombinant Human Active Protein Kinase

HGNC Symbol: BMPR1B

Synonyms: ALK6, CDw293

Product No.: 1667-0000-1

Lot: 001

Description: Human BMPR1B, C-terminal fragment, amino acids Y_{149}-L_{502} (as in NCBI/Protein entry NP_001194.1), N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: BMPR1B Lot 001, was confirmed as BMPR1B by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 63,387 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

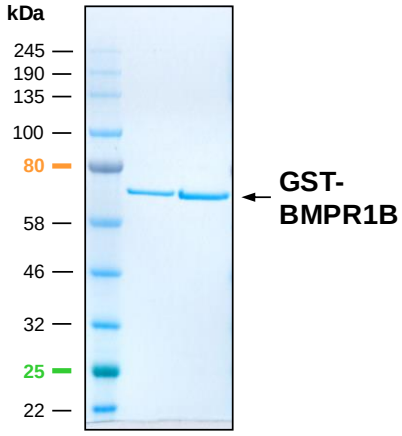
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

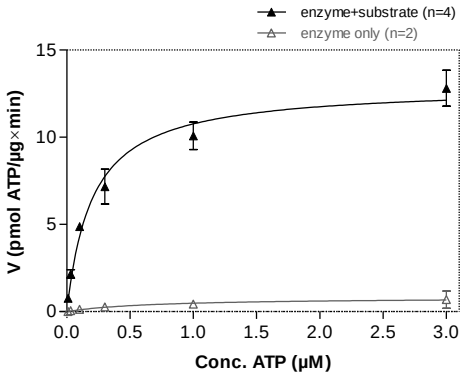
Protein concentration: 0.674 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 13 pmol/µg × min
ATP-K_M: 0.2 µM

BMPR1B Lot 001:
Coomassie stain



BMPR1B Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- 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: Casein 40 µg/ml
  Kinase: 0.2 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology:
BMPR1B 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™ BMPR1B

Product No.: 1667-0000-1

GST-BMPR1B Recombinant Fusion Protein Amino Acid Sequence

```
   1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID   60
  61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV  120
 121  DFLSKLPEML KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK  180
 181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG  240
 241  PYKRQETRPR YSIGLEQDET YIPPGESLRD LIEQSQSSGS GSGLPLLVQR TIAKQIQMVK  300
 301  QIGKGRYGEV WMGKWRGEKV AVKVFFTTEE ASWFRETEIY QTVLMRHENI LGFIAADIKG  360
 361  TGSWTQLYLI TDYHENGSLY DYLKSTTLDA KSMLKLAYSS VSGLCHLHTE IFSTQGKPAI  420
 421  AHRDLKSKNI LVKKNGTCCI ADLGLAVKFI SDTNEVDIPP NTRVGTKRYM PPEVLDESLN  480
 481  RNHFQSYIMA DMYSFGLILW EVARRCVSGG IVEEYQLPYH DLVPSDPSYE DMREIVCIKK  540
 541  LRPSFPNRWS SDECLRQMGK LMTECWAHNP ASRLTALRVK KTLAKMSESQ DIKL        600
```

1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: BMPR1B fragment

BMPR1B wt[1] Amino Acid Sequence

```
1    MLLRSAGKLN VGTKKEDGES TAPTPRPKVL RCKCHHHCPE DSVNNICSTD GYCFTMIEED   60
61   DSGLPVVTSG CLGLEGSDFQ CRDTPIPHQR RSIECCTERN ECNKDLHPTL PPLKNRDFVD  120
121  GPIHHRALLI SVTVCSLLLV LIILFCYFRY KRQETRPRYS IGLEQDETYI PPGESLRDLI  180
181  EQSQSSGSGS GLPLLVQRTI AKQIQMVKQI GKGRYGEVWM GKWRGEKVAV KVFFTTEEAS  240
241  WFRETEIYQT VLMRHENILG FIAADIKGTG SWTQLYLITD YHENGSLYDY LKSTTLDAKS  300
301  MLKLAYSSVS GLCHLHTEIF STQGKPAIAH RDLKSKNILV KKNGTCCIAD LGLAVKFISD  360
361  TNEVDIPPNT RVGTKRYMPP EVLDESLNRN HFQSYIMADM YSFGLILWEV ARRCVSGGIV  420
421  EEYQLPYHDL VPSDPSYEDM REIVCIKKLR PSFPNRWSSD ECLRQMGKLM TECWAHNPAS  480
481  RLTALRVKKT LAKMSESQDI KL                                           540
```

blue: BMPR1B sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_001194.1

Recombinant Proteins