Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ BRK

protein tyrosine kinase 6

Recombinant Human Active Protein Kinase

HGNC Symbol: PTK6

Synonyms: n/a

Product No.: 0181-0000-1

Lot: 004

Description: Human BRK, full length, amino acids M_1-T_{451} (as in NCBI/Protein entry NP_005966.1), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: BRK Lot 004, was confirmed as BRK by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 81,790 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

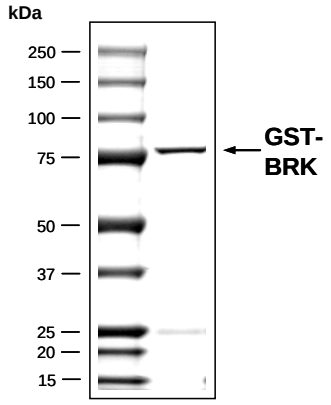
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

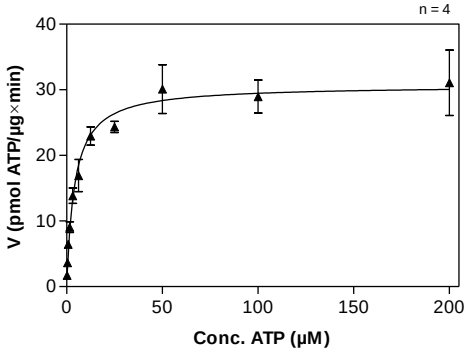
Biochemical Parameters:
Specific kinase activity (P_i transfer): 31 pmol/µg × min
ATP-K_M: 4.1 µM

BRK Lot 004:
Coomassie stain



2.0 µg GST-BRK

BRK Lot 004:
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: Poly(Glu/Tyr)$_{4:1}$ 20 µg/ml
  Kinase: 1 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology:
BRK Lot 004 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

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GST-BRK 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 GIYARGIQAS MVSRDQAHLG PKYVGLWDFK SRTDEELSFR AGDVFHVARK 300
 301 EEQWWWATLL DEAGGAVAQG YVPHNYLAER ETVESEPWFF GYISRSEAVR RLQAEGNATG 360
 361 AFLIRVSEKP SADYVLSVRD TQAVRHYKIW RRAGGRLHLN EAVSFLSLPE LVNYHRAQSL 420
 421 SHGLRLAAPC RKHEPEPLPH WDDWERPREE FTLCRKLGSG YFGEVFEGLW KDRVQVAIKV 480
 481 ISRDNLLHQQ MLQSEIQAMK KLRHKHILAL YAVVSVGDPV YIITELMAKG SLLELLRDSD 540
 541 EKVLPVSELL DIAWQVAEGM CYLESQNYIH RDLAARNILV GENTLCKVGD FGLARLIKED 600
 601 VYLSHDHNIP YKWTAPEALS RGHYSTKSDV WSFGILLHEM FSRGQVPYPG MSNHEAFLRV 660
 661 DAGYRMPCPL ECPPSVHKLM LTCWCRDPEQ RPCFKALRER LSSFTSYENP T          720
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: BRK

BRK wt[1] Amino Acid Sequence

```
   1 MVSRDQAHLG PKYVGLWDFK SRTDEELSFR AGDVFHVARK EEQWWWATLL DEAGGAVAQG  60
  61 YVPHNYLAER ETVESEPWFF GCISRSEAVR RLQAEGNATG AFLIRVSEKP SADYVLSVRD 120
 121 TQAVRHYKIW RRAGGRLHLN EAVSFLSLPE LVNYHRAQSL SHGLRLAAPC RKHEPEPLPH 180
 181 WDDWERPREE FTLCRKLGSG YFGEVFEGLW KDRVQVAIKV ISRDNLLHQQ MLQSEIQAMK 240
 241 KLRHKHILAL YAVVSVGDPV YIITELMAKG SLLELLRDSD EKVLPVSELL DIAWQVAEGM 300
 301 CYLESQNYIH RDLAARNILV GENTLCKVGD FGLARLIKED VYLSHDHNIP YKWTAPEALS 360
 361 RGHYSTKSDV WSFGILLHEM FSRGQVPYPG MSNHEAFLRV DAGYRMPCPL ECPPSVHKLM 420
 421 LTCWCRDPEQ RPCFKALRER LSSFTSYENP T                               480
```

blue: kinase sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_005966.1

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