

Certificate of Analysis

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 M₁-T₄₅₁ (as in NCBI/Protein entry NP_005966.1), N-terminal GST-HIS₆ 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: Baculovirus infected Sf9 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

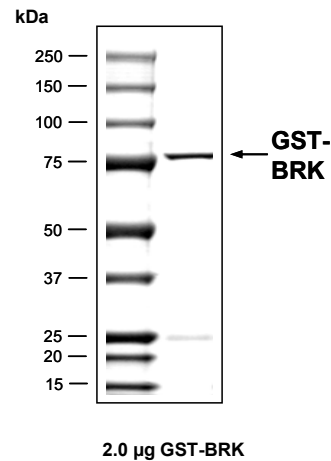
ATP-K_M: 4.1 µM

Additional assay technology: BRK Lot 004

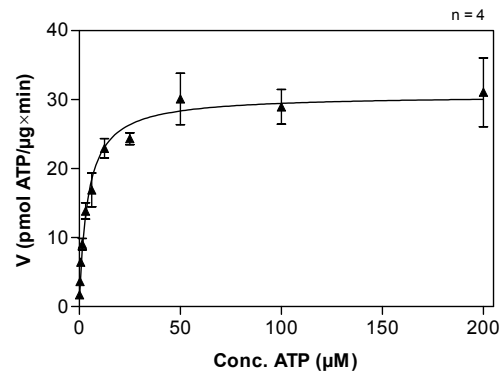
was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega. ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details



BRK Lot 004: Coomassie stain



BRK Lot 004: 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 MgCl₂
 - 3 mM MnCl₂
 - 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: 1.0 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

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BRK 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	KRIEAIQID	KYLKSSKYIA	WPLQGQATF	GGGDHPPKSD	PMG HHHHHH HG	RRRASVAAGI	240
241	LVPRGS PGLD	GIYARGIQAS	MVSRDQAHLG	PKYVGLWDFK	SRTDEELSFR	AGDVFHVARK	300
301	EEQWWATLL	DEAGGAVAQG	YVPHNYLAER	ETVESEPFFF	GYISRSEAVR	RLQAEGNATG	360
361	AFLIRVSEKP	SADYVLSVRD	TQAVRHYKIW	RRAGGRLHLN	EAVSFLSLPE	LVNYHRAQSL	420
421	SHGLRLAAPC	RKHEPEPLPH	WDDWERPREE	FTLCRKLGS	YFGEVFEGLW	KDRVQVAIKV	480
481	ISRDNLLHQQ	MLQSEIQAMK	KLRHKHILAL	YAVVSVGDPV	YIITELMAKG	SLELLLRDSD	540
541	EKVLVSELL	DAIWQVAEGM	CYLESQNYIH	RDLAARNILV	GENTLCKVGD	FGLARLIKED	600
601	VYLSHDHNIP	YKWTAPEALS	RGHYSTKSDV	WSFGILLHEM	FSRGQVPYPG	MSNHEAFLRV	660
661	DAGYRMPCPL	ECPPSVHKLM	LTCWCRDPEQ	RPCFKALRER	LSSFTSYENP	T	720

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**:BRK **boxed**:variation from RefSeq

BRK wt ¹ Amino Acid Sequence							
1	MVSRDQAHLG	PKYVGLWDFK	SRTDEELSFR	AGDVFHVARK	EEQWWATLL	DEAGGAVAQG	60
61	YVPHNYLAER	ETVESEPFFF	GISRSEAVR	RLQAEGNATG	AFLIRVSEKP	SADYVLSVRD	120
121	TQAVRHYKIW	RRAGGRLHLN	EAVSFLSLPE	LVNYHRAQSL	SHGLRLAAPC	RKHEPEPLPH	180
181	WDDWERPREE	FTLCRKLGS	YFGEVFEGLW	KDRVQVAIKV	ISRDNLLHQQ	MLQSEIQAMK	240
241	KLRHKHILAL	YAVVSVGDPV	YIITELMAKG	SLELLLRDSD	EKVLVSELL	DAIWQVAEGM	300
301	CYLESQNYIH	RDLAARNILV	GENTLCKVGD	FGLARLIKED	VYLSHDHNIP	YKWTAPEALS	360
361	RGHYSTKSDV	WSFGILLHEM	FSRGQVPYPG	MSNHEAFLRV	DAGYRMPCPL	ECPPSVHKLM	420
421	LTCWCRDPEQ	RPCFKALRER	LSSFTSYENP	T			480

blue: BRK sequence expressed in fusion protein **Red**: variant in fusion protein

¹NCBI/Protein accession number NP_005966.1