Certificate of Analysis

REACTION BIOLOGY

ProQinase™ BLK

BLK proto-oncogene, Src family tyrosine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: BLK

Synonyms: MODY11, MGC10442

Product No.: 0448-0000-1

Lot: 001

Description: Human BLK, full length, amino acids M1-P505 (as in NCBI/Protein entry NP_001706.2), N-terminal GST-HIS6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

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

Theoretical $MW_{Fusion\ Protein}$: 87,102 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

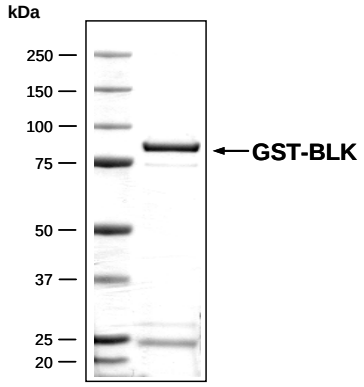
Storage buffer: 50 mM TRIS-HCl pH 8.0, 100 mM NaCl, 5 mM DTT, 4 mM reduced glutathione, 20 % glycerol

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

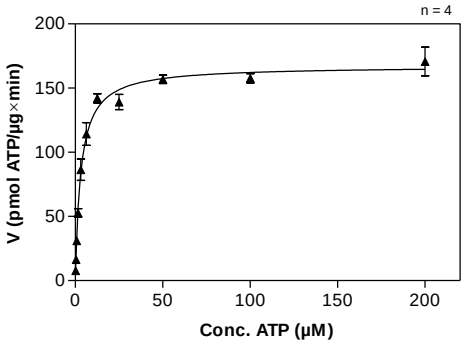
Biochemical Parameters:
Specific kinase activity (P_i transfer): 167 pmol/µg × min
ATP-K_M: 3.1 µM

BLK Lot 001:
Coomassie stain



2.0 µg GST-BLK

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

Additional assay technology:
BLK 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

Recombinant Proteins

ProQinase™ BLK

Product No.: 0448-0000-1

GST-BLK 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 GICSRMGLVS SKKPDKEKPI KEKDKGQWSP LKVSAQDKDA PPLPPLVVFN 300
301 HLTPPPPDEH LDEDKHFVVA LYDYTAMNDR DLQMLKGEKL QVLKGTGDWW LARSLVTGRE 360
361 GYVPSNFVAR VESLEMERWF FRSQGRKEAE RQLLAPINKA GSFLIRESET NKGAFSLSVK 420
421 DVTTQGELIK HYKIRCLDEG GYYISPRITF PSLQALVQHY SKKGDGLCQR LTLPCVRPAP 480
481 QNPWAQDEWE IPRQSLRLVR KLGSGQFGEV WMGYYKNNMK VAIKTLKEGT MSPEAFLGEA 540
541 NVMKALQHER LVRLYAVVTK EPIYIVTEYM ARGCLLDFLK TDEGSRLSLP RLIDMSAQIA 600
601 EGMAYIERMN SIHRDLRAAN ILVSEALCCK IADFGLARII DSEYTAQEGA KFPIKWTAPE 660
661 AIHFGVFTIK ADVWSFGVLL MEVVTYGRVP YPGMSNPEVI RNLERGYRMP RPDTCPPELY 720
721 RGVIAECWRS RPEERPTFEF LQSVLEDFYT ATERQYELQP                       780
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1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: BLK

BLK wt[1] Amino Acid Sequence

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  1 MGLVSSKKPD KEKPIKEKDK GQWSPLKVSA QDKDAPPLPP LVVFNHLTPP PPDEHLDEDK  60
 61 HFVVALYDYT AMNDRDLQML KGEKLQVLKG TGDWWLARSL VTGREGYVPS NFVARVESLE 120
121 MERWFFRSQG RKEAERQLLA PINKAGSFLI RESETNKGAF SLSVKDVTTQ GELIKHYKIR 180
181 CLDEGGYYIS PRITFPSLQA LVQHYSKKGD GLCQRLTLPC VRPAPQNPWA QDEWEIPRQS 240
241 LRLVRKLGSG QFGEVWMGYY KNNMKVAIKT LKEGTMSPEA FLGEANVMKA LQHERLVRLY 300
301 AVVTKEPIYI VTEYMARGCL LDFLKTDEGS RLSLPRLIDM SAQIAEGMAY IERMNSIHRD 360
361 LRAANILVSE ALCCKIADFG LARIIDSEYT AQEGAKFPIK WTAPEAIHFG VFTIKADVWS 420
421 FGVLLMEVVT YGRVPYPGMS NPEVIRNLER GYRMPRPDTC PPELYRGVIA ECWRSRPEER 480
481 PTFEFLQSVL EDFYTATERQ YELQP                                       540
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blue: BLK sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_001706.2

Recombinant Proteins