

Certificate of Analysis

BLK

B lymphoid 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: 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: 0.120 µg/µl

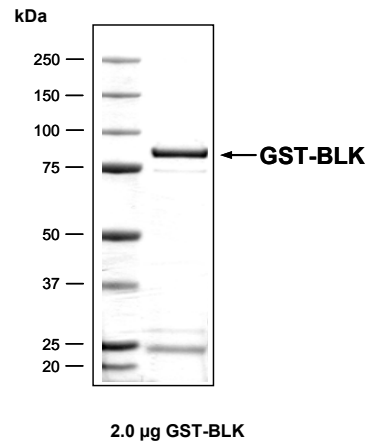
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

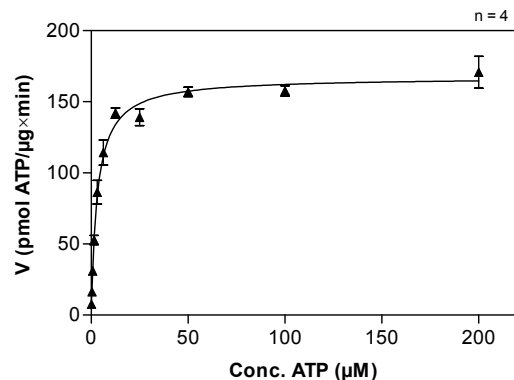
Specific kinase activity (P_i transfer): 167 pmol/µg × min

ATP-K_M: 3.1 µM

BLK Lot 001: Coomassie stain



BLK 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 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: 4.0 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: BLK Lot 001

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



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BLK Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RRRASVAAGI	240
241	LVPRGSPGLD	GICSRMGLVS	SKKPDKEKPI	KEKDKGQWSP	LKVSAQDKDA	PPLPPLVVFN	300
301	HLTPPPPDEH	LDEDKHFVVA	LYDYTAMNDR	DLQMLKGEKL	QVLKGTGDWW	LARSLVTGRE	360
361	GYVPSNFVAR	VESLEMERWF	FRSQGRKEAE	RQLLAPINKA	GSFLIRESET	NKGAFLSVK	420
421	DVTTQGELIK	HYKIRCLDEG	GYIISPRITF	PSLQALVQHY	SKKGDGLCQR	LTLPCVRPAP	480
481	QNPWAQDEWE	IPRQSLRLVR	KLGSQGFGEV	WMGYKNNMK	VAIKTLKEGT	MSPEAFLGEA	540
541	NVMKALQHER	LVRLYAVVTK	EPIYIVTEYM	ARGCLLDFLK	TDEGSRLSLP	RLIDMSAQIA	600
601	EGMAYIERMN	SIHRDLRAAN	ILVSEALCCK	IADFGLARII	DSEYTAQEGA	KFPKWTAPE	660
661	AIHFGVFTIK	ADVWSFGVLL	MEVVTYGRVP	YPGMSNPEVI	RNLERGYRMP	RPDTCPPELY	720
721	RGVIAECWRS	RPEERPTFEF	LQSVLEDFYT	ATERQYELQP			780

1-218: GST **Red:** HIS6-tag **Pink:** Thrombin cleavage site **blue:**BLK

BLK wt ¹ Amino Acid Sequence							
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	QFGEVWMGY	KNNMKVAIKT	LKEGTMSPEA	FLGEANVMKA	LQHERLVRLY	300
301	AVVTKEPIYI	VTEYMARGCL	LDLFLTDEGS	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

blue: BLK sequence expressed in fusion protein

¹NCBI/Protein accession number NP_001706.2