Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active BLK [1 - 505]

Enzyme description:- BLK [1 - 505]

Clone number:- DU 35196

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal GST

Purification method:- GSH Agarose

Calculated molecular mass:-
Monoisotopic 84, 476.22 daltons
Average Mass 84, 530.66 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.58

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 10 mM DTT

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc

Substrate:-
POLY (Glu, Tyr, 4:1) Final concentration: 1 mg/ml

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Clone Data Sheet

BLK [1 - 505]

Protein BLK [1 - 505]

Clone number DU 35186

Species Human

Accession number NM_001715.3

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNK
KFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKE
RAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKM
FEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKL
VCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSD
LEVLFQGPLGS**MGLVSSKKPDKEKPIKEKDKGQWSPLKVSAQDK**
DAPPLPPLVVFNHLTPPPPDEHLDEDKHFVVALYDYTAMNDRDL
QMLKGEKLQVLKGTGDWWLARSLVTGREGYVPSNFVARVESLEM
ERWFFRSQGRKEAERQLLAPINKAGSFLIRESETNKGAFSLSVK
DVTTQGELIKHYKIRCLDEGGYYISPRITFPSLQALVQHYSKKG
DGLCQRLTLPCVRPAPQNPWAQDEWEIPRQSLRLVRKLGSGQFG
EVWMGYYKNNMKVAIKTLKEGTMSPEAFLGEANVMKALQHERLV
RLYAVVTKEPIYIVTEYMARGCLLDFLKTDEGSRLSLPRLIDMS
AQIAEGMAYIERMNSIHRDLRAANILVSEALCCKIADFGLARII
DSEYTAQEGAKFPIKWTAPEAIHFGVFTIKADVWSFGVLLMEVV
TYGRVPYPGMSNPEVIRNLERGYRMPRPDTCPPELYRGVIAECW
RSRPEERPTFEFLQSVLEDFYTATERQYELQP

Native sequence Amino acids M1 – P505 (end residue) of human BLK. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 - 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Bam*H1 and *Not*1sites of pFastBac Dual.

Nucleotide sequence of insert

ggatccATGGGGCTGGTAAGTAGCAAAAAGCCGGACAAGGAAAAG
CCGATCAAAGAGAAGGACAAGGGCCAATGGAGCCCCCTGAAGGTC
AGCGCCCAAGACAAGGACGCCCCGCCACTGCCGCCCCTGGTTGTC
TTCAACCACCTTACTCCTCCACCGCCCGATGAACACCTGGATGAA
GACAAGCATTTCGTGGTGGCTCTGTATGACTACACCGCTATGAAT
GATCGGGACCTGCAGATGCTGAAGGGGGAGAAGCTACAGGTCCTG
AAGGGAACTGGAGACTGGTGGCTGGCCAGGTCACTCGTCACAGGA
AGAGAAGGCTATGTGCCCAGCAACTTTGTGGCCCGAGTGGAGAGC
CTGGAAATGGAAAGGTGGTTCTTTAGATCACAGGGTCGGAAGGAG
GCTGAGAGGCAGCTTCTTGCTCCAATCAACAAGGCCGGCTCCTTT
CTTATCAGAGAGAGTGAAACCAACAAAGGTGCCTTCTCCCTGTCT
GTGAAGGATGTCACCACCCAGGGGGAGCTGATCAAGCACTATAAG
ATCCGCTGCCTGGATGAAGGGGGCTACTACATCTCCCCCCGGATC
ACCTTCCCCTCGCTCCAGGCCCTGGTGCAGCACTATTCTAAGAAG
GGGGATGGTCTATGCCAGAGGCTGACCCTGCCCTGTGTGCGCCCG
GCCCCGCAGAATCCCTGGGCCCAGGATGAATGGGAGATCCCCCGG
CAGTCTCTCAGGCTGGTCAGGAAACTCGGGTCTGGACAATTCGGC
GAAGTCTGGATGGGTTACTACAAAAACAACATGAAGGTGGCCATT
AAGACGCTGAAGGAGGGAACCATGTCTCCAGAAGCCTTTCTGGGT
GAGGCCAACGTGATGAAGGCTCTGCAGCACGAGCGGCTGGTCCGA
CTCTACGCAGTGGTCACCAAGGAGCCCATCTACATTGTCACCGAG
TACATGGCCAGAGGATGCCTGCTGGATTTCCTGAAGACAGATGAA
GGGAGCAGATTGTCACTCCCAAGGCTGATTGACATGTCGGCGCAG
ATTGCTGAAGGGATGGCATACATTGAGCGCATGAATTCCATCCAC
CGCGACCTGCGGGCGGCCAACATCCTGGTGTCTGAGGCCTTGTGC
TGCAAAATTGCTGATTTTGGCTTGGCTCGAATCATCGACAGTGAA
TACACGGCCCAAGAGGGGGCCAAGTTCCCCATCAAGTGGACAGCC
CCGGAAGCCATCCACTTCGGGGTCTTCACCATCAAAGCAGACGTG
TGGTCGTTTGGAGTCCTCCTGATGGAAGTTGTCACTTATGGGCGG
GTGCCATACCCAGGGATGAGCAACCCCGAGGTCATCCGCAACCTG
GAGCGCGGCTACCGCATGCCGCGCCCCGACACCTGCCCGCCCGAG
CTGTACCGCGGCGTCATCGCCGAGTGCTGGCGCAGCCGGCCCGAG
GAGCGGCCCACCTTCGAGTTCCTGCAGTCGGTGCTGGAGGACTTC
TACACGGCCACCGAGCGGCAGTACGAGCTGCAGCCCtaggcggcc
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