Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PKB gamma [117 - 479]

Enzyme description:- PKB gamma [117 – 479]

Clone number:- DU 62934

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Cobalt Agarose

Calculated molecular mass:-
Monoisotopic 45, 355.23 daltons
Average Mass 45, 384.48 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.76

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 10 mM DTT, 10 mM Magnesium acetate

Substrate:-
GRPRTSSFAEGKK Final concentration: 300 uM

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Clone Data Sheet

PKB gamma [117 - 479]

Protein	PKB gamma [117 - 479]
Clone number	DU 62934
Species	Human
Accession number	NM_005465.7
Tags	N-terminal His6
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGRS**MNCSPTSQIDNIGEEEMDA STTHHKRKTMNDFDYLKLLGKGTFGKVILVREKASGKYYAMKILKKEV IIAKDEVAHTLTESRVLKNTRHPFLTSLKYSFQTKDRLCFVMEYVNGG ELFFHLSRERVFSEDRTRFYGAEIVSALDYLHSGKIVYRDLKLENLML DKDGHIKITDFGLCKEGITDAATMKTFCGTPEYLAPEVLEDNDYGRAV DWWGLGVVMYEMMCGRLPFYNQDHEKLFELILMEDIKFPRTLSSDAKS LLSGLLIKDPNKRLGGGPDDAKEIMRHSFFSGVNWQDVYDKKLVPPFK PQVTSETDTRYFDEEFTAQTITITPPEKYDEDGMDCMDNERRPHFPQF SYSASGRE**

Native sequence	Amino acids M117 – E479 (end) of human PKB gamma. Residue M30 of the fusion protein is equivalent to M117 of the native enzyme. The His6 tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 – 24
Cloning sites	*Bgl*II *Eco*R1 into *Bam*H1 *Eco*R1 sites into pFastBac HTb

Nucleotide sequence of insert

ATGAATTGTAGTCCAACTTCACAAATTGATAATATAGGAGAGGAAGAG
ATGGATGCCTCTACAACCCATCATAAAAGAAAGACAATGAATGATTTT
GACTATTTGAAACTACTAGGTAAAGGCACTTTTGGGAAAGTTATTTTG
GTTCGAGAGAAGGCAAGTGGAAAATACTATGCTATGAAGATTCTGAAG
AAAGAAGTCATTATTGCAAAGGATGAAGTGGCACACACTCTAACTGAA
AGCAGAGTATTAAAGAACACTAGACATCCCTTTTTAACATCCTTGAAA
TATTCCTTCCAGACAAAAGACCGTTTGTGTTTTGTGATGGAATATGTT
AATGGGGGCGAGCTGTTTTTCCATTTGTCGAGAGAGCGGGTGTTCTCT
GAGGACCGCACACGTTTCTATGGTGCAGAAATTGTCTCTGCCTTGGAC
TATCTACATTCCGGAAAGATTGTGTACCGTGATCTCAAGTTGGAGAAT
CTAATGCTGGACAAAGATGGCCACATAAAAATTACAGATTTTGGACTT
TGCAAAGAAGGGATCACAGATGCAGCCACCATGAAGACATTCTGTGGC
ACTCCAGAATATCTGGCACCAGAGGTGTTAGAAGATAATGACTATGGC
CGAGCAGTAGACTGGTGGGGCCTAGGGGTTGTCATGTATGAAATGATG
TGTGGGAGGTTACCTTTCTACAACCAGGACCATGAGAAACTTTTTGAA
TTAATATTAATGGAAGACATTAAATTTCCTCGAACACTCTCTTCAGAT
GCAAAATCATTGCTTTCAGGGCTCTTGATAAAGGATCCAAATAAACGC
CTTGGTGGAGGACCAGATGATGCAAAAGAAATTATGAGACACAGTTTC
TTCTCTGGAGTAAACTGGCAAGATGTATATGATAAAAAGCTTGTACCT
CCTTTTAAACCTCAAGTAACATCTGAGACAGATACTAGATATTTTGAT
GAAGAATTTACAGCTCAGACTATTACAATAACACCACCTGAAAAATAT
GATGAGGATGGTATGGACTGCATGGACAATGAGAGGCGGCCGCATTTC
CCTCAATTTTCCTACTCTGCAAGTGGACGAGAAtaagaattc