Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PKB gamma [1 - 479]

Enzyme description:- PKB gamma [1 – 479]

Clone number:- DU 62933

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Cobalt Agarose

Calculated molecular mass:-
Monoisotopic 59, 108.22 daltons
Average Mass 59, 146.15 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.78

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 [1 - 479]

Protein	PKB gamma [1 - 479]
Clone number	DU 62933
Species	Human
Accession number	NM_005465.7
Tags	N-terminal His6
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MSDVTIVKEGWVQKRGEYIK NWRPRYFLLKTDGSFIGYKEKPQDVDLPYPLNNFSVAKCQLMKTERPK PNTFIIRCLQWTTVIERTFHVDTPEEREEWTEAIQAVADRLQRQEEER MNCSPTSQIDNIGEEEMDASTTHHKRKTMNDFDYLKLLGKGTFGKVIL VREKASGKYYAMKILKKEVIIAKDEVAHTLTESRVLKNTRHPFLTSLK YSFQTKDRLCFVMEYVNGGELFFHLSRERVFSEDRTRFYGAEIVSALD YLHSGKIVYRDLKLENLMLDKDGHIKITDFGLCKEGITDAATMKTFCG TPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRLPFYNQDHEKLFE LILMEDIKFPRTLSSDAKSLLSGLLIKDPNKRLGGGPDDAKEIMRHSF FSGVNWQDVYDKKLVPPFKPQVTSETDTRYFDEEFTAQTITITPPEKY DEDGMDCMDNERRPHFPQFSYSASGRE**
Native sequence	Amino acids M1 – E479 (end) of human PKB gamma. Residue M29 of the fusion protein is equivalent to M1 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

ggatctATGAGCGATGTTACCATTGTGAAAGAAGGTTGGGTTCAGAAG
AGGGGAGAATATATAAAAAACTGGAGGCCAAGATACTTCCTTTTGAAG
ACAGATGGCTCATTCATAGGATATAAAGAGAAACCTCAAGATGTGGAT
TTACCTTATCCCCTCAACAACTTTTCAGTGGCAAAATGCCAGTTAATG
AAAACAGAACGACCAAAGCCAAACACATTTATAATCAGATGTCTCCAG
TGGACTACTGTTATAGAGAGAACATTTCATGTGGATACTCCAGAGGAA
AGGGAAGAATGGACAGAAGCTATCCAGGCTGTAGCAGACAGACTGCAG
AGGCAAGAAGAGGAGAGAATGAATTGTAGTCCAACTTCACAAATTGAT
AATATAGGAGAGGAAGAGATGGATGCCTCTACAACCCATCATAAAAGA
AAGACAATGAATGATTTTGACTATTTGAAACTACTAGGTAAAGGCACT
TTTGGGAAAGTTATTTTGGTCCGAGAGAAGGCAAGTGGAAAATACTAT
GCTATGAAGATTCTGAAGAAAGAAGTCATTATTGCAAAGGATGAAGTG
GCACACACTCTAACTGAAAGCAGAGTATTAAAGAACACTAGACATCCC
TTTTTAACATCCTTGAAATATTCCTTCCAGACAAAAGACCGTTTGTGT
TTTGTGATGGAATATGTTAATGGGGGCGAGCTGTTTTTCCATTTGTCG
AGAGAGCGGGTGTTCTCTGAGGACCGCACACGTTTCTATGGTGCAGAA
ATTGTCTCTGCCTTGGACTATCTACATTCCGGAAAGATTGTGTACCGT
GATCTCAAGTTGGAGAATCTAATGCTGGACAAAGATGGCCACATAAAA
ATTACAGATTTTGGACTTTGCAAAGAAGGGATCACAGATGCAGCCACC
ATGAAGACATTCTGTGGCACTCCAGAATATCTGGCACCAGAGGTGTTA
GAAGATAATGACTATGGCCGAGCAGTAGACTGGTGGGGCCTAGGGGTT
GTCATGTATGAAATGATGTGTGGGAGGTTACCTTTCTACAACCAGGAC
CATGAGAAACTTTTTGAATTAATATTAATGGAAGACATTAAATTTCCT
CGAACACTCTCTTCAGATGCAAAATCATTGCTTTCAGGGCTCTTGATA
AAGGATCCAAATAAACGCCTTGGTGGAGGACCAGATGATGCAAAAGAA
ATTATGAGACACAGTTTCTTCTCTGGAGTAAACTGGCAAGATGTATAT
GATAAAAAGCTTGTACCTCCTTTTAAACCTCAAGTAACATCTGAGACA
GATACTAGATATTTTGATGAAGAATTTACAGCTCAGACTATTACAATA
ACACCACCTGAAAAATATGATGAGGATGGTATGGACTGCATGGACAAT
GAGAGGCGGCCGCATTTCCCTCAATTTTCCTACTCTGCAAGTGGACGA
GAAtaagaattc