University of Dundee

Standard Operating Procedure

Preparation of active PKB beta S474D [120 - 481] ΔPH domain

Enzyme description:- PKB beta S474D [120 - 481] ΔPH domain

Clone Number:- DU 1851

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 3 mg/L

Calculated molecular mass:- 43, 470 daltons

Purity:- >80 %

Activation protocol:-
PKB beta (4 μM) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM magnesium acetate, 0.1 mM ATP with 3.3 μg/ml GST-PDK1 [DU 954] for 30 min at 30 °C. Following activation, PKB beta is re-purified by Ni^{2+}-NTA agarose chromatography.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
Crosstide [GRPRTSSFAEG] Final concentration: 30 μM

Specific Activity Range:- 250 - 500 U/mg

University of Dundee

CLONE DATA SHEET - PKB beta S474D [120 - 481] ΔPH domain

Protein	PKB beta [120 - 481] ΔPH domain
Clone number	DU 1851
Species	Human
Accession no	NM_001626
Tags	N-terminal His(6)
Baculovirus expressed protein	MAHHHHHHARVPRGS**MDYKCGSPSDSSTTEEMEVAVSKARAKVTMNDF DYLKLLGKGTFGKVILVREKATGRYYAMKILRKEVIIAKDEVAHTVTE SRVLQNTRHPFLTALKYAFQTHDRLCFVMEYANGGELFFHLSRERVFT EERARFYGAEIVSALEYLHSRDVVYRDIKLENLMLDKDGHIKITDFGL CKEGISDGATMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMM CGRLPFYNQDHERLFELILMEEIRFPRTLSPEAKSLLAGLLKKDPKQR LGGGPSDAKEVMEHRFFLSINWQDVVQKKLLPPFKPQVTSEVDTRYFD DEFTAQSITITPPDRYDSLGLLELDQRTHFPQFDYSASIRE**
Native sequence	Amino acids M120 – E377 (end) of human PKB beta. Residue M16 of the fusion protein is equivalent to M120 of the native enzyme. The enzyme has a S474D mutation to mimic phosphorylation of the PDK2 site. Residue S474 is equilivalent to D370 of the fusion protein. The His(6) tag is located at residues 3 - 8
Protease cleavage	Thrombin (RVPRGS) residues 10 – 15

Cloning sites

Nucleotide sequence of insert

ATGGACTACAAGTGTGGCTCCCCCAGTGACTCCTCCACGACTGAGGAG
ATGGAAGTGGCGGTCAGCAAGGCACGGGCTAAAGTGACCATGAATGAC
TTCGACTATCTCAAACTCCTTGGCAAGGGAACCTTTGGCAAAGTCATC
CTGGTGCGGGAGAAGGCCACTGGCCGCTACTACGCCATGAAGATCCTG
CGAAAGGAAGTCATCATTGCCAAGGATGAAGTCGCTCACACAGTCACC
GAGAGCCGGGTCCTCCAGAACACCAGGCACCCGTTCCTCACTGCGCTG
AAGTATGCCTTCCAGACCCACGACCGCCTGTGCTTTGTGATGGAGTAT
GCCAACGGGGGTGAGCTGTTCTTCCACCTGTCCCGGGAGCGTGTCTTC
ACAGAGGAGCGGGCCCGGTTTTATGGTGCAGAGATTGTCTCGGCTCTT
GAGTACTTGCACTCGCGGGACGTGGTATACCGCGACATCAAGCTGGAA
AACCTCATGCTGGACAAAGATGGCCACATCAAGATCACTGACTTTGGC
CTCTGCAAAGAGGGCATCAGTGACGGGGCCACCATGAAAACCTTCTGT
GGGACCCCGGAGTACCTGGCGCCTGAGGTGCTGGAGGACAATGACTAT
GGCCGGGCCGTGGACTGGTGGGGGCTGGGTGTGGTCATGTACGAGATG
ATGTGCGGCCGCCTGCCCTTCTACAACCAGGACCACGAGCGCCTCTTC
GAGCTCATCCTCATGGAAGAGATCCGCTTCCCGCGCACGCTCAGCCCC
GAGGCCAAGTCCCTGCTTGCTGGGCTGCTTAAGAAGGACCCCAAGCAG
AGGCTTGGTGGGGGGCCCAGCGATGCCAAGGAGGTCATGGAGCACAGG
TTCTTCCTCAGCATCAACTGGCAGGACGTGGTCCAGAAGAAGCTCCTG
CCACCCTTCAAACCTCAGGTCACGTCCGAGGTCGACACAAGGTACTTC
GATGATGAATTTACCGCCCAGTCCATCACAATCACACCCCCTGACCGC
TATGACAGCCTGGGCTTACTGGAGCTGGACCAGCGGACCCACTTCCCC
CAGTTCGACTACTCGGCCAGCATCCGCGAGtga