Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active PKB alpha S473D [118 – 480] Δ PH domain

Enzyme description:- PKB alpha S473D [118 - 480] Δ PH domain

Clone number:- DU 1850

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 2-3 mg/L

Calculated molecular mass:- 45, 103 daltons

Purity:- >80 %

Activation protocol:-
PKB alpha (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 alpha 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

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Clone Data Sheet

PKB alpha S473D [118 – 480] ΔPH domain

Protein	PKB alpha S473D [118 - 480] ΔPH domain
Clone number	DU 1850
Species	Human
Accession no	BC000479
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MDFRSGSPSDNSGAE EMEVSLAKPKHRVTMNEFEYLKLLGKGTFGKVILVKEKATGRY YAMKILKKEVIVAKDEVAHTLTENRVLQNSRHPFLTALKYSFQ THDRLCFVMEYANGGELFFHLSRERVFSEDRARFYGAEIVSAL DYLHSEKNVVYRDLKLENLMLDKDGHIKITDFGLCKEGIKDGA TMKTFCGTPEYLAPEVLEDNDYGRAVDWWGLGVVMYEMMCGRL PFYNQDHEKLFELILMEEIRFPRTLGPEAKSLLSGLLKKDPKQ RLGGGSEDAKEIMQHRFFAGIVWQHVYEKKLSPPFKPQVTSET DTRYFDEEFTAQMITITPPDQDDSMECVDSERRPHFPQFDYSA SGTA**
Native sequence	Amino acids M118 - A480 (end) of human PKB alpha. Residue M29 of the fusion protein is equivalent to M118 of the native enzyme. The enzyme has a S473D mutation to mimic phosphorylation of the PDK2 site. Residue S473 is equilivalent to D384 of the fusion protein. The His(6) tag is located at residues 5 - 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 – 24
Cloning sites	*Bam*HI and *Kpn*I sites of pFastBAC HTb.

Nucleotide sequence of insert

ATGGACTTCCGGTCGGGCTCACCCAGTGACAACTCAGGGGCTGAAGAG
ATGGAGGTGTCCCTGGCCAAGCCCAAGCACCGCGTGACCATGAACGAG
TTTGAGTACCTGAAGCTGCTGGGCAAGGGCACTTTCGGCAAGGTGATC
CTGGTGAAGGAGAAGGCCACAGGCCGCTACTACGCCATGAAGATCCTC
AAGAAGGAAGTCATCGTGGCCAAGGACGAGGTGGCCCACACACTCACC
GAGAACCGCGTCCTGCAGAACTCCAGGCACCCCTTCCTCACAGCCCTG
AAGTACTCTTTCCAGACCCACGACCGCCTCTGCTTTGTCATGGAGTAC
GCCAACGGGGGCGAGCTGTTCTTCCACCTGTCCCGGGAGCGTGTGTTC
TCCGAGGACCGGGCCCGCTTCTATGGCGCTGAGATTGTGTCAGCCCTG
GACTACCTGCACTCGGAGAAGAACGTGGTGTACCGGGACCTCAAGCTG
GAGAACCTCATGCTGGACAAGGACGGGCACATTAAGATCACAGACTTC
GGGCTGTGCAAGGAGGGGATCAAGGACGGTGCCACCATGAAGACCTTT
TGCGGCACACCTGAGTACCTGGCCCCCGAGGTGCTGGAGGACAATGAC
TACGGCCGTGCAGTGGACTGGTGGGGGCTGGGCGTGGTCATGTACGAG
ATGATGTGCGGTCGCCTGCCCTTCTACAACCAGGACCATGAGAAGCTT
TTTGAGCTCATCCTCATGGAGGAGATCCGCTTCCCGCGCACGCTTGGT
CCCGAGGCCAAGTCCTTGCTTTCAGGGCTGCTCAAGAAGGACCCCAAG
CAGAGGCTTGGCGGGGGCTCCGAGGACGCCAAGGAGATCATGCAGCAT
CGCTTCTTTGCCGGTATCGTGTGGCAGCACGTGTACGAGAAGAAGCTC
AGCCCACCCTTCAAGCCCCAGGTCACGTCGGAGACTGACACCAGGTAT
TTTGATGAGGAGTTCACGGCCCAGATGATCACCATCACACCACCTGAC
CAAGATGACAGCATGGAGTGTGTGGACAGCGAGCGCAGGCCCCACTTC
CCCCAGTTCGACTACTCGGCCAGCGGCACGGCCtga