PKB alpha (S473D) [6His-tagged]

Kinase

Alternate Names: AKT1, RAC-alpha serine/threonine-protein kinase, RAC-PK-alpha

Cat. No.	**66-0017-050**	Quantity:	50 µg
Lot. No.	**30296**	Storage:	-70˚C

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NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

PKB alpha (PKB α; AKT1) is one of three closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3) which may be alternatively named PKB α, PKB β, and PKB γ, respectively. Together, they regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis. This is mediated through serine and/or threonine phosphorylation of a range of downstream substrates (Kumar *et al.*, 2013). Cloning of the gene was first described by Staal *et al.* (1987). PKB alpha is a member of the most frequently activated proliferation and survival pathway in cancer. The activation of PKB alpha is driven by membrane localization, which is in turn initiated by the binding of the pleckstrin homology (PH) domain to phosphatidylinositol-3,4,5-trisphosphate or phosphatidylinositol-3,4-bisphosphate, followed by phosphorylation of the regulatory amino acids serine 473 (Ser-473) and threonine 308 (Thr-308) on PKB alpha (Kumar and Purohit, 2013). PKB alpha seems to have a crucial but passive role in oncogenesis and acts as an indirect intermediary between mutated upstream regulatory proteins and downstream signalling molecules (Kumar and Purohit, 2013). PKB alpha is involved in the phosphorylation of members of the FOXO factors (Forkhead family of transcription factors), leading to binding of 14-3-3 proteins and cytoplasmic localisation (Rena *et al.*, 1999). Unregulated activation of the PKB pathway is a prominent fea-

Continued on page 2

Physical Characteristics

Species: human

Source: baculovirus expression vector system

Quantity: 50 µg

Concentration: 0.39 mg/ml

Formulation: 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% ß-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~45.1kDa

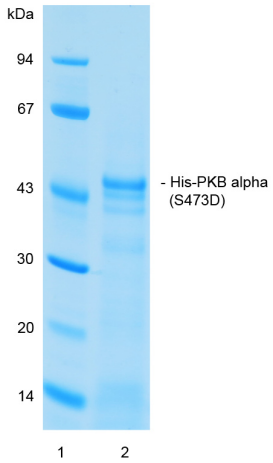
Purity: >80% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg His-PKB alpha (S473D)



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of His-PKB alpha (S473D) was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. His-PKB alpha (S473D) was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of CROSStide substrate (30 µM) and [γ-32P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate.The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

His-PKB alpha (S473D) specific activity:
665.7 Units/mg (259.6 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: CROSStide (GRPRTSSFAEG)

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Kinase

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Background

Continued from page 1

ture of many human cancers and PKB alpha is overexpressed or activated in all major cancers. For these reasons, PKB alpha is considered an attractive target for cancer therapy (Wang *et al.*, 2011).

References:

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Kumar A and Purohit R (2013) Cancer associated E17K mutation causes rapid conformational drift in AKT1 pleckstrin homology (PH) domain. *PLoS One* **8**, e64364.

Kumar A, Rajendran V, Sethumadhavan R and Purohit R (2013) AKT kinase pathway: a leading target in cancer research. *Scientific World Journal* **2013**, 756134.

Rena G, Guo S, Cichy SC, Unterman TG and Cohen P (1999) Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. *J Biol Chem* **274**, 17179-17183.

Staal SP (1987) Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. *Proc Natl Acad Sci U S A* **84**, 5034-5037.

Wang P, Zhang L, Hao Q and Zhao G (2011) Developments in selective small molecule ATP-targeting the serine/threonine kinase Akt/PKB. *Mini Rev Med Chem* **11**, 1093-1107.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSYYHHHHHHDYDIPTTENLYFQGAMGS***M***D
FRSGSPSDNSGAEEMEVSLAKPKHRVTM
NEFEYLKLLGKGTFGKVILVKEKATGRYYAM
KILKKEVIVAKDEVAHTLTENRVLQNSRHPFL
TALKYSFQTHDRLCFVMEYANGGELFFHLSR
ERVFSEDRARFYGAEIVSALDYLHSEKNV
VYRDLKLENLMLDKDGHIKITDFGLCKEGIKD
GATMKTFCGTPEYLAPEVLEDNDYGRAVD
WWGLGVVMYEMMCGRLPFYNQDHEKLFE
LILMEEIRFPRTLGPEAKSLLSGLLKKDPKQR
LGGGSEDAKEIMQHRFFAGIVWQHVYEKKL
SPPFKPQVTSETDTRYFDEEFTAQMITITPP
DQDDSMECVDSERRPHFPQFDYSASGTA

Tag (**bold text**): N-terminal 6His
Protease cleavage site: TEV (ENLYFQ▼)
PKB alpha (S473D) (regular text): Start ***bold italics*** (amino acid residues 118-480)
PKB alpha has a S473D mutation to mimic the activation of the enzyme through phosphorylation of Ser473 by PDK2.
Accession number: NP_001014431

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