PKB alpha (human; residues 1-147), pAb

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

Cat. No.	**68-0030-100**	Quantity:	100 µg
Lot. No.	**30269**	Storage:	-20˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS |

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. PKB alpha (AKT1) is one of 3 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

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human PKB alpha (residues 1-147) [6His-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects PKB alpha at ~45 kDa

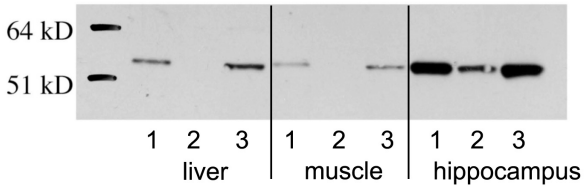
Reactivity: human; other species not tested

Stability/Storage: 12 months at -20˚C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
not tested

Immunoprecipitation:
10 µg/mg



Lane 1: Pellet, Lane 2: Supernatent, Lane 3: Input.

Immunoprecipitation Assay:
Immunoprecipitation was performed from various cell types (0.2 mg of cell extract) using a mixture of 2 µg of each anti-PKB antibody (anti-PKB alpha Cat# 68-0030-100; anti-PKB beta Cat# 68-0031-100; anti-PKB gamma Cat# 68-0032-100). The following samples were analysed by SDS-PAGE/Western blotting (probing with a commercially available anti-PKB antibody); the input cell extract (Lane 3), the supernatent (Lane 2) and the immunoprecipitate (Lane 1; pellet). PKB was not detectable in the supernatant (except for the sample derived from hippocampus).

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

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Lot-specific COA version tracker: v1.0.0

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Cat. No.	**68-0030-100**	Quantity:	100 µg
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CERTIFICATE OF ANALYSIS

Background

Continued from page 1

phosphatidylinositol-3,4-bisphosphate, followed by phosphorylation of the regulatory amino acids serine 473 (Ser-473) and threonine 308 (Thr-308) (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 feature of many human cancers and PKB alpha is overexpressed or activated in all major cancers. For these reasons, PKB alpha is considered as an attractive target for cancer therapy (Wang *et al.*, 2011).

Antibody Production:

Anti-PKB alpha (human) polyclonal antibody was raised in sheep against PKB alpha (residues 1-147 of human PKB alpha). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti- PKB alpha pAbs from the sheep serum using a 6His-tagged antigen-agarose column. Anti-PKB alpha (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

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 USA* **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.

Application References:

Walker KS, Deak M, Paterson A, Hudson K, Cohen P and Alessi DR (1998) Activation of protein kinase B beta and gamma isoforms by insulin *in vivo* and by 3-phosphoinositide-dependent protein kinase-1 *in vitro*: comparison with protein kinase B alpha. *Biochemical J* **331** (Pt 1), 299-308.

Sommer EM, Dry H, Cross D, Guichard S, Davies BR and Alessi DR (2013) Elevated SGK1 predicts resistance of breast cancer cells to Akt inhibitors. *Biochemical J* **452**, 499-508.

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