PKB gamma (human; residues 116-127), pAb

Alternate Names: AKT3, RAC-gamma serine/threonine-protein kinase, Protein kinase Akt-3, Protein kinase B gamma

Cat. No.	**68-0032-100**	Quantity:	100 µg
Lot. No.	**30271**	Storage:	-20˚C

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CERTIFICATE OF ANALYSIS | **Page 1 of 2**

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 post-translational 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 gamma (AKT3) 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 Brodbeck *et al.* (1999). An example of the interplay between ubiquitylation and phosphorylation has been highlighted in a paper studying the role of PKB gamma in atherosclerosis. Akt3 specifically inhibits macrophage cholesteryl ester accumulation and foam cell formation, a critical early event in athero-

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: PKB gamma (residues 116-127) [RMNCSPTSQIDN]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects PKB gamma at ~56 kDa

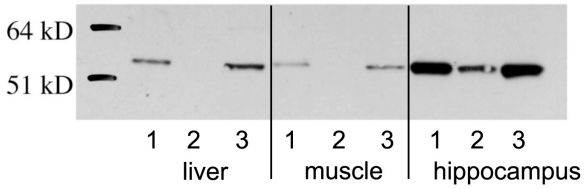
Reactivity: human; other species not tested

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

Research Applications and Quality Assurance

Western Immunoblotting:
not tested

Immunoprecipitation:
use 10 µg/mg of cell extract



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).

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

genesis. Mechanistically, Akt3 suppresses foam cell formation by reducing lipoprotein uptake and phosphorylating ACAT-1 (acyl-CoA:cholesterol acyltransferase) promoting degradation via the ubiquitin-proteasome pathway. The finding of selective regulation of ACAT-1 by Akt3 adds new possibilities for pharmacological regulation of ACAT-1, which may be of interest beyond the field of atherosclerosis (Ding *et al.*, 2012).

Antibody Production:

Anti-PKB gamma (human) polyclonal antibody was raised in sheep against PKB gamma (residues 116-127 of human PKB gamma). 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 gamma pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-PKB gamma (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Brodbeck D, Cron P and Hemmings BA (1999) A human protein kinase Bgamma with regulatory phosphorylation sites in the activation loop and in the C-terminal hydrophobic domain. *J Biol Chem* **274**, 9133-9136.

Ding L, Biswas S, Morton RE, Smith JD, Hay N, Byzova TV, *et al.* (2012) Akt3 deficiency in macrophages promotes foam cell formation and atherosclerosis in mice. *Cell Metab* **15**, 861-872.

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

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. *Biochem J* **331** (Pt 1), 299-308.

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