PKB beta (mouse; residues 455-469), pAb

Alternate Names: AKT2, RAC-beta serine/threonine-protein kinase, Protein kinase Akt-2, Protein kinase B beta

Cat. No.	**68-0031-100**	Quantity:	100 µg
Lot. No.	**30270**	Storage:	-20˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

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 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 beta (AKT2) 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). An example of such interplay between phosphorylation and ubiquitylation has been highlighted in a recent study showing how the E3 ligase TRAF6 can regulate Akt2 ubiquitylation and activation, suggesting that TRAF6 may even promote Akt phosphorylation by way of Akt2 ubiquitylation. These findings help towards the understanding for a pivotal role of ubiquitylation in endotoxemia-induced myocardial injury. Furthermore, the

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: PKB beta (residues 455-469) [RYDSLDPLELDQRTH]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects PKB beta at ~56 kDa

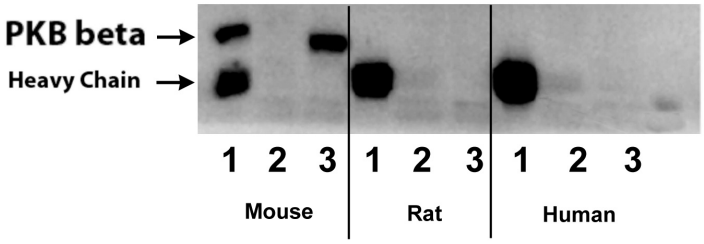
Reactivity: mouse; does not detect rat or human PKB beta

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

Research Applications and Quality Assurance

Western Immunoblotting:
use 1 µg/ml

Immunoprecipitation:
use 10 µg/mg of cell extract



Lane 1: Pellet, Lane 2: Supernatant, Lane 3: Input

Western Blotting Analysis:
Immunoprecipitation was performed from mouse, rat and human lysates using a commercially available anti-PKB beta antibody. PKB beta was subsequently detected by Western Blot using 1 µg/ml anti-PKB beta antibody (Cat# 68-0031-100). In order to show that all PKB was immunoprecipitated from the input cell extract (Lane 3), a Western Blot was carried out using anti-PKB antibody (Cat# 68-0031-100) on the supernatent (Lane 2) and the immunoprecipitate (Lane 1; pellet). PKB beta was not detectable in the mouse supernatant and showed no crossreactivity with rat or human PKB beta.

'Heavy Chain' denotes the IgG heavy chain of the immunoprecipitating antibody which is detected by the immunoblotting anti-PKB antibody (Cat# 68-0031-100).

Continued on page 2

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

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

PKB beta (mouse; residues 455-469), pAb

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

Cat. No.	**68-0031-100**	Quantity:	100 µg
Lot. No.	**30270**	Storage:	-20˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS |

Background

Continued from page 1

study has revealed the clinical value of Akt2 and TRAF6 – as well as ubiquitylation – as potential therapeutic targets in the management of heart failure in sepsis (Yang *et al.*, 2009; Zhang *et al.*, 2014).

Antibody Production:
Anti-PKB beta (mouse) polyclonal antibody was raised in sheep against PKB beta (residues 455-469 of mouse PKB beta). 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 beta pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-PKB beta (mouse) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

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

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.

Yang WL, Wang J, Chan CH, Lee SW, Campos AD, Lamothe B, *et al.* (2009) The E3 ligase TRAF6 regulates Akt ubiquitination and activation. *Science* **325**, 1134-1138.

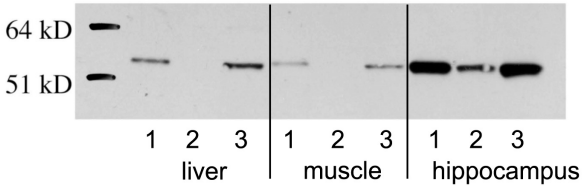
Zhang Y, Xu X, Ceylan-Isik AF, Dong M, Pei Z, Li Y, *et al.* (2014) Ablation of Akt2 protects against lipopolysaccharide-induced cardiac dysfunction: Role of Akt ubiquitination E3 ligase TRAF6. *J Mol Cell Cardiol* **74C**, 76-87.

Application Reference:

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.

Research Applications and Quality Assurance

Continued from page 1



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

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0