

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. The serumand glucocorticoid-inducible protein kinase (SGK) family is made up of three isoforms, SGK1, 2, and 3, that are phosphatidylinositide-3-kinase (PI3-K)-dependent, serine/ threonine kinases, with similar substrate specificity to protein kinase B (PKB). Consequently, the SGK family also regulates similar cell processes to the PKB kinases, including cell proliferation and survival (Bruhn et al., 2013). Cloning of the SGK3 gene was first described by Kobayashi et al. (1999). To date, little is known about factors affecting the stability and degradation of SGK2 and SGK3. A recent publication has shown that, in contrast to SGK1, SGK3 is stable and that the stability results from a hydrophobic motif in the C terminus and constitutive association of SGK3 with an

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SGK3 (human; full length), pAb

Alternate Names: Serine/threonine-protein kinase Sgk3, Serum/glucocorticoid-regulated kinase 3

Cat. No.	68-0036-100
Lot. No.	30275

Quantity: Storage: 100 µg -20°С

FOR RESEARCH USE ONLY

CERTIFICATE OF ANALYSIS

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NOT FOR USE IN HUMANS

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human SGK3 (residues 1-300) [GST-tagged]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects SGK3 at ~57.1 kDa

Reactivity: human; other species not tested

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

Research Applications and Quality Assurance

Western Immunoblotting: use 1 µg/ml

Immunoprecipitation: use 5 µg/mg of cell extract



Western Blotting Analysis:

HEK293 cells were transfected with vectors expressing HA-SGK3 or HA-tag as a control. The cells were then lysed and the lysates denatured in SDS and subjected to SDS-PAGE on 8% gels. Western Blotting was carried out with 1 μ g/ml anti-SGK3 antibody (Cat# 68-0036-100) or a commercially available anti-HA antibody.

Immunoprecipitation Assay:

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Immunoprecipitation was performed from HEK293 cells over-expressing HA-tagged SGK3 (1 mg) using 5 µg of anti-SGK3 antibody (Cat# 68-0036-100). SGK3 was subsequently detected by Western Blot using a commercially available anti-HA antibody.

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Lot-specific COA version tracker: v1.0.0



Background

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Hsp90-Cdc37 chaperone complex, both of which protect the kinase from ubiquitin E3 ligase CHIP-mediated proteasomal degradation (Wang *et al.*, 2014).

Antibody Production:

Anti-SGK3 (human) polyclonal antibody was raised in sheep against SGK3 (residues 1-300 of human SGK3). 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-SGK3 pAbs from the sheep serum using an antigenagarose column followed by depletion of any anti-GST antibodies using a GSTagarose column. Anti-SGK3 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Bruhn MA, Pearson RB, Hannan RD and Sheppard KE (2013) AKTindependent PI3-K signaling in cancer - emerging role for SGK3. *Cancer Manag Res* 5, 281-292.

Kobayashi T, Deak M, Morrice N and Cohen P (1999) Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. *Biochem J* **344** Pt 1, 189-197.

Wang Y, Xu W, Zhou D, Neckers L and Chen S (2014) Coordinated regulation of serum- and glucocorticoid-inducible kinase 3 by a C-terminal hydrophobic motif and Hsp90-Cdc37 chaperone complex. *J Biol Chem* **289**, 4815-4826.

Application Reference:

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. *Biochem J* **452**, 499-508.

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