





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 serum- and 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 gene for SGK2 was first described by Kobayashi et al. (1999). An example of the interplay between ubiquitylation and phosphorylation can be shown by SGK2 - plus SGK1, SGK3 and PKB - ability to regulate the glutamate transporter EAAT2. These kinases bind to and phosphorylate the ubiquitin E3 ligase Nedd4-2, hence de-

Continued on page 2

SGK2 (human; residues 333-346), pAb

Alternate Names: Serine/threonine-protein kinase Sgk2, Serum/glucocorticoid-regulated kinase 2

Cat. No. 68-0035-100 Quantity: 100 µg Lot. No. 30274 -20°C Storage:

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on

shipping

Source: sheep polyclonal antibody

Immunogen: human SGK2 (residues 333-346) [KSIGCTPDTVASSS]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered

Specificity: detects SGK2 at ~ 47.6

Reactivity: human; other species not

tested

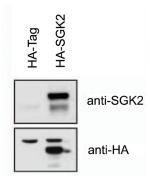
Stability/Storage: 12 months at -20°C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:

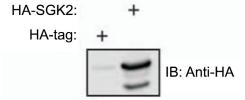
use 1 µg/ml

Immunoprecipitation: use 5 µg/mg of cell extract



Western Blotting Analysis:

HEK293 cells were transfected with vectors expressing HA-SGK2 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 µg/ml anti-SGK2 antibody (Cat# 68-0035-100) or a commercially available anti-HA antibody.



Immunoprecipitation Assay:

Immunoprecipitation was performed from HEK293 cells over-expressing HA-tagged SGK2 (1 mg) using 5 µg of anti-SGK2 antibody (Cat# 68-0035-100). SGK2 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







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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

Continued from page 1

creasing the affinity of the E3 ligase to its target proteins and thus, stimulating the activity and cell surface expression of EAAT2. The mechanism is likely to participate in the regulation of neuronal excitability whereby defective expression of the EAAT2 transporter results in neuroexcitotoxicity that may contribute to neuronal disorders such as amyotrophic lateral sclerosis (ALS) (Boehmer et al., 2006).

Antibody Production:

Anti-SGK2 (human) polyclonal antibody was raised in sheep against SGK2 (residues 333-346 of human SGK2). 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-SGK2 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-SGK2 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Boehmer C, Palmada M, Rajamanickam J, Schniepp R, Amara S and Lang F (2006) Post-translational regulation of EAAT2 function by co-expressed ubiquitin ligase Nedd4-2 is impacted by SGK kinases, J Neurochem 97, 911-921,

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.



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