SGK1 (S422D) [6His-tagged]

Kinase

Alternate Names: Serine/threonine-protein kinase Sgk1, Serum/glucocorticoid-regulated kinase 1

Cat. No. **66-0020-050**
Lot. No. **30299**

Quantity: 50 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

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 was first described by Webster *et al.* (1993). SGK1 is activated by insulin, growth factors and oxidative stress via PI3-K, 3-phosphoinositide-dependent kinase PDK1 and mTOR. Mechanisms employed by SGK1 in transport regulation include direct phosphorylation of target transport proteins, phosphorylation and thus activation of other transport regulating kinases, stabilisation of membrane proteins by phosphorylation and thus inactivation of the ubiquitin

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Physical Characteristics

Species: human

Source: baculovirus expression vector system

Quantity: 50 µg

Concentration: 0.69 mg/ml

Formulation: 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% ß-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~45.5 kDa

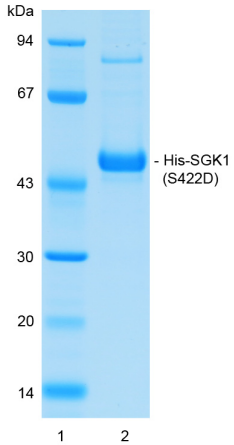
Purity: >90% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg His-SGK1 (S422D)



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of His-SGK1 (S422D) was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. His-SGK1 (S422D) was incubated for 10 minutes at 30˚C in kinase reaction buffer in the presence of CROSStide substrate (30 µM) and [γ-32P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate.The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

His-SGK1 (S422D) specific activity:
1088 Units/mg (750.8 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: CROSStide (GRPRTSSFAEG)

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

Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

E3 ligase NEDD4-2, as well as stimulation of transport protein expression by up-regulating transcription factors (e.g. nuclear factor kappa-B (NFκB)) and by fostering of protein translation. Moreover, excessive SGK1 activity has been shown to contribute to the pathophysiology of hypertension, obesity, diabetes, thrombosis, stroke, inflammation, autoimmune disease, fibrosis and tumour growth (Lang *et al.*, 2014)

References:

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

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Lang F, Stournaras C and Alesutan I (2014) Regulation of transport across cell membranes by the serum- and glucocorticoid-inducible kinase SGK1. *Mol Membr Biol* **31**, 29-36.

Park J, Leong ML, Buse P, Maiyar AC, Firestone GL et al. (1999) Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. *EMBO J* **11**, 3024-33.

Webster MK, Goya L, Ge Y, Maiyar AC and Firestone GL (1993) Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. *Mol Cell Biol* **13**, 2031-2040.

Physical Characteristics

Continued from page 1

Protein Sequence:

M S Y Y H H H H H H D Y D I P T T E N
LYFQGAMG***I***SQPQEPELMNANPSPPPSPSQQ
INLGPSSNPHAKPSDFHFLKVIGKGSFGKVL
LARHKAEEVFYAVKVLQKKAILKKKEEKHIM
SERNVLLKNVKHPFLVGLHFSFQTADKLYFV
LDYINGGELFYHLQRERCFLEPRARFYAAE
IASALGYLHSLNIVYRDLKPENILLDSQGHIV
LTDFGLCKENIEHNSTTSTFCGTPEYLAPE
VLHKQPYDRTVDWWCLGAVLYEMLYGLPP
FYSRNTAEMYDNILNKPLQLKPNITNSARHL
LEGLLQKDRTKRLGAKDDFMEIKSHVFFSLIN
WDDLINKKITPPFNPNVSGPNDLRHFDPEFT
EEPVPNSIGKSPDSVLVTASVKEAAEAFL
GFDYAPPTDSFL

Tag (**bold text**): N-terminal 6His
Protease cleavage site: TEV (ENLYFQ▼)
SGK1 (regular text): Start ***bold italics*** (amino acid residues 60-431).
SGK1 has a S422D mutation to mimic the activation of the enzyme through phosphorylation of Ser422 by PDK2 (Park *et al.*, 1999)
Accession number: NP_005618

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Lot-specific COA version tracker: v1.0.0