BioVision Incorporated
980 Linda Vista Avenue Mountain View, CA 94043 USA Phone: (650)428-0236 Fax: (650)428-0336

Product Specification

SGK1(Δ60aa), active
(Recombinant human protein, N-terminal 60 amino acid-deleted, expressed in Sf 9 cells)

Catalog #: 7748-5
Lot #: _____
Aliquot size: 5 µg protein in 50 µl
Specific activity: 65 nmol/min/mg

Quality Control Analysis
Activity assessment
SGK1 protein (~100 ng/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl diluted proteins were used to phosphorylate the Akt/SGK substrate (RPRAATF) in the following assay condition:

10 µl diluted SGK1 protein
5 µl Akt/SGK substrate (1 mg/ml stock)
5 µl water
5 µl [^{32}P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [^{32}P] ATP, were incubated at 30° C and the reaction started by the addition of [^{32}P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

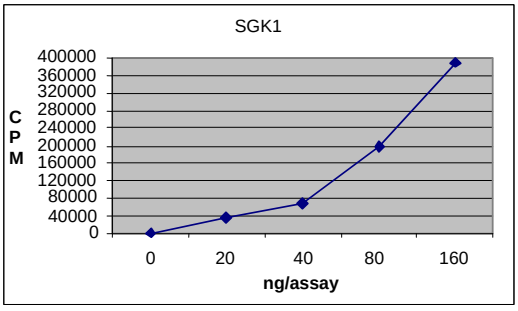

Fig. 1 SGK1 activity assay

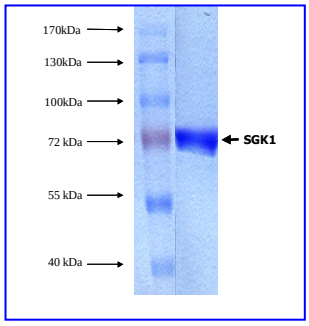

Fig. 2 SGK1 protein gel

Purity assessment
2 µg of SGK1 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >95% purity of the SGK1 product, and the band was at ~73 kDa (Fig. 2)

Product Description
Recombinant N-truncated (missing N-terminal 60 amino acides), N-terminal GST-fusion human SGK1 was purified on an affinity column from baculovirus-infected Sf9 cells.
The gene accession number is NM_005627.
This material is sold for research purposes only.

Specific Activity
65 nmol phosphate incorporated into Akt/SGK substrate per minute per mg protein at 30° C for 15 minutes using a final concentration of 50 µM ATP (0.83 µCi/assay).

Formulation
Recombinant protein in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

Storage and Stability
Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

Scientific Background
SGK1 is a member of the serum- and glucocorticoid-induced protein kinase that is activated *in vitro* by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and *in vivo* in response to signals that activate phosphatidylinositol (PI) 3-kinase (1). SGK1, mRNA is expressed in all tissues and the levels of SGK1 mRNA is increased by stimulation with serum or dexamethasone.
SGK1 promotes cell survival by phosphorylating and inactivating FKHRL1. SGK and Akt display differences with respect to the efficacy with which they phosphorylate the three regulatory sites on FKHRL1. While both kinases can phosphorylate Thr-32, SGK1 displays a marked preference for Ser-315 whereas Akt favors Ser-253. These findings suggest that SGK1 and Akt may coordinately regulate the function of FKHRL1 by phosphorylating this transcription factor at distinct sites. Like PKB, SGK1 preferentially phosphorylate Ser and Thr residues that lie in Arg-Xaa-Arg-Xaa-Xaa-Ser/Thr motifs. SGK1 gene has recently been identified as an important aldosterone-induced protein kinase that mediates trafficking of the renal epithelial Na(+) channel (ENaC) to the cell membrane. Thus, SGK1 is an appealing candidate for blood pressure regulation and possibly essential hypertension (3).

References
1. Kobayashi T, Deak M, Morrice N, Cohen P. Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. Biochem J. 1999 Nov 15;344 Pt 1:189-97.
2. Brunet A, Park J, Tran H, Hu LS, Hemmings BA, Greenberg ME. Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). Mol Cell Biol. 2001 Feb;21(3):952-65.
3. Busjahn A, Aydin A, Uhlmann R, Krasko C, Bahring S, Szelestei T, Feng Y, Dahm S, Sharma AM, Luft FC, Lang F. Serum- and glucocorticoid-regulated kinase (SGK1) gene and blood pressure. Hypertension. 2002 Sep;40(3):256-60.