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Product Specification

SGK2, active
(Full-length recombinant protein expressed in Sf 9 cells)

Catalog #: 7749
Lot #:
Aliquot size: 5 µg protein in 50 µl
Specific activity: 82 nmol/min/mg

Quality Control Analysis
Activity assessment
SGK2 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 and 40ng/µl BSA), 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 SGK2 protein
10 µl Akt/SGK substrate peptide(1 mg/ml stock)
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.

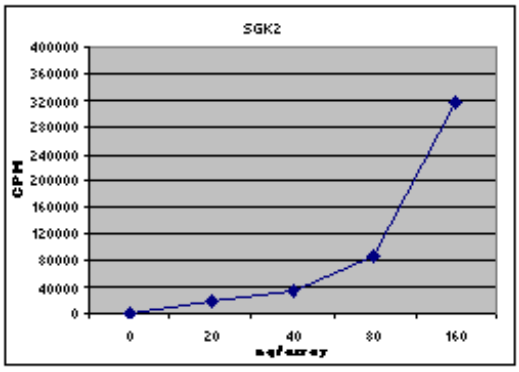

Fig. 1 SGK2 activity assay

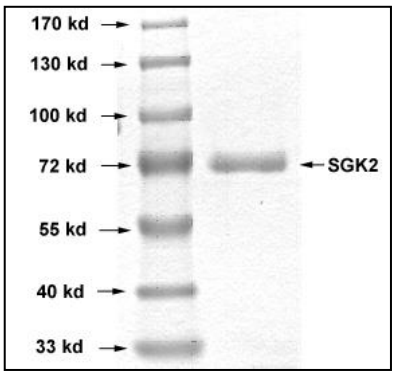

Fig. 2 SGK2 protein gel

Purity assessment
1 µg of SGK2 protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the SGK2 product, and the band was at ~71 kDa (Fig. 2)

Product Description

Recombinant full length human SGK2 containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.
The gene accession number is NM_170693.
This material is sold for research purposes only.

Specific Activity
82 nmol phosphate incorporated into Akt/SGK substrate peptide (RPRAATF) 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
SGK2 is a member of the serum- and glucocorticoid-induced kinases (SGK) which are serine-threonine kinases and belong to the "AGC" kinase subfamily, which includes protein kinases A, G, and C, and its catalytic domain is most similar to protein kinase B (PKB). SGK1 was originally identified as a glucocorticoid-sensitive gene and subsequently, the two homologous kinases SGK2 and SGK3 have been cloned, being products of distinct genes, which are differentially expressed and share 80% identity in amino acid sequence in their catalytic domains . SGK2, like the other two isoforms SGK1 and SGK3, is stimulated by insulin and insulin-like growth factor-1 (IGF-1), and has been shown to enhance Na(+)/K(+)-ATPase activity in a variety of cells. In addition, SGK2 mimics the function of SGK1 and SGK3 and participate in the regulation of renal epithelial Na(+) channel ENaC activity.

SGK2 is activated by phosphorylation in response to signals that stimulate phosphatidylinositol 3-kinase by a huge number of extracellular signals. The phosphorylation of SGK2 is mediated by 3-phosphoinositide-dependent protein kinase 1 (PDK1) and other protein kinases that have yet to be identified. The substrate specificity of SGK isoforms superficially resembles that of PKB in that serine and threonine residues lying in Arg-Xaa-Arg-Xaa-Xaa-Ser/Thr sequences (where Xaa is a variable amino acid) are phosphorylated. However, although they may have some substrates in common, evidence is emerging that SGKs and PKB phosphorylate distinct proteins and have different functions in vivo. In particular, SGKs play an important role in activating certain potassium, sodium, and chloride channels, suggesting an involvement in the regulation of processes such as cell survival, neuronal excitability, and renal sodium excretion. Moreover, sustained high levels of SGK protein and activity may contribute to conditions such as hypertension and diabetic nephropathy.