

SGK2 Kinase

✓ 5 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: Purified recombinant full length human SGK2 (Met1-Cys367) kinase, supplied as a GST fusion protein.

Background: Serum and glucocorticoid-inducible kinase (SGK), a serine/threonine kinase, is a close relative of Akt (1). SGK is rapidly induced in response to a variety of stimuli, including serum, glucocorticoid, follicle stimulating hormone, osmotic shock and mineralocorticoids. SGK activation can be accomplished via HGF PI3K-dependent pathways and by integrin-mediated PI3K-independent pathways (2,3). Induction and activation of SGK has been implicated in activating the modulation of antiapoptotic and cell cycle regulation (4-6). SGK also plays an important role in activating certain potassium, sodium and chloride channels, suggesting its involvement in the regulation of processes such as cell survival, neuronal excitability and renal sodium excretion (2). SGK is negatively regulated by ubiquitin modification and proteasome degradation (7).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human SGK2 (Met1-Cys367) (GenBank Accession No. NM_170693) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-SGK2 fusion protein is 67 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. SGK2 kinase activity was determined using a radiometric assay [Fig.2].

Background References:

- (1) Webster, M.K. et al. (1993) *Mol. Cell. Biol.* 13, 2031–2040.
- (2) Kobayashi, T. and Cohen, P. (1999) *Biochem. J.* 339, 319–328.
- (3) Park, J. et al. (1999) *EMBO J.* 18, 3024–3033.
- (4) Brunet, A. et al. (2001) *Mol. Cell. Biol.* 21, 952–965.
- (5) Mikosz, C.A. et al. (2001) *J. Biol. Chem.* 276, 16649–16654.
- (6) Hayashi, M. et al. (2001) *J. Biol. Chem.* 276, 8631–8634.
- (7) Brickley, D.R. et al. (2002) *J. Biol. Chem.* 277, 43064–43070.

Storage: Enzyme is supplied in 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, 7 mM glutathione. Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

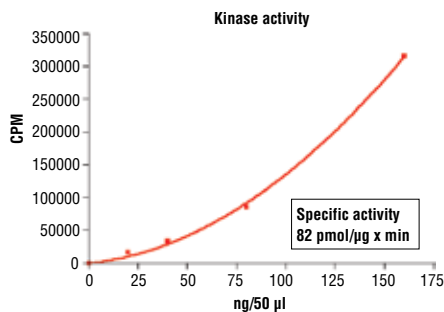


Figure 2. SGK2 kinase activity was measured in a radiometric assay using the following reaction conditions: 4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 4 mM MgCl₂, 0.05 mM DTT, 50 µM ATP, 0.6 µM BSA, Substrate: Akt/SGK substrate, 400 ng/µL and recombinant SGK2: variable.

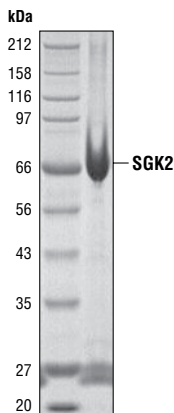


Figure 1. The purity of the GST-SGK2 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Protocol for SGK2 Kinase Assay

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

- 1. Kinase Buffer (10X)**
40 mM MOPS, pH 7.2
25 mM β -glycerophosphate
10 mM EGTA
4 mM $MgCl_2$
0.5 mM DTT
6 μ M BSA
- 2.** ATP (10 mM) #9804
- 3.** ^{32}P - γ ATP
- 4.** Akt/SGK Substrate (RPRAATF)

B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250 μ M ATP.
- 2.** Dilute [^{32}P] ATP to 0.16 μ Ci/ μ l [^{32}P] ATP with 250 μ M ATP solution.
- 3.** Transfer enzyme from $-80^\circ C$ to ice. Allow enzyme to thaw on ice.
- 4.** Dilute SGK2 protein (100 ng/ μ l concentration) to 20 ng/ μ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10 μ l diluted SGK2 kinase solution, 10 μ l Akt/SGK substrate (1 μ g/ μ l) and 5 μ l 0.16 μ Ci/ μ l [^{32}P] ATP solution.

Final Assay Conditions

- 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
 - 0.6 μ M BSA
 - 400 ng/ μ l Akt/SGK substrate
- 6.** After 15 minutes terminate reaction by spotting 20 μ l of the reaction mixture onto phosphocellulose P81 paper.
 - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
 - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
 - 9.** Count samples in a scintillation counter.

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