# Rabbit (polyclonal) Anti-SGK1 [pS$^{422}$] Phosphospecific Antibody, Unconjugated

## PRODUCT ANALYSIS SHEET

**Catalog Number:** 44-1264G (10 mini-blot size)

**Lot Number:** See product label

**Volume:** 100 µL

**Form of Antibody:** Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without $Mg^{2+}$ and $Ca^{2+}$), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier.

**Preservative:** 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)

**Purification:** Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively pre-adsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated SGK1. The final product is generated by affinity chromatography using a SGK1-derived peptide that is phosphorylated at serine 422.

**Immunogen:** The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human SGK1 that contains serine 422.

**Target Summary:** Serum and glucocorticoid-regulated kinase 1 (SGK1) is a ~50 kDa serine/threonine kinase that plays a central role in a variety of epithelial, cardiac and neuronal transport systems and in regulating homeostasis and metabolism. SGK1 is transcriptionally regulated by serum, glucocorticoids and mineralocorticoids. SGK1 activity is regulated by phosphorylation. SGK1 is phosphorylated at several sites including serine 256 and threonine 422, PDK-1 and PDK-2 sites respectively. Phosphorylation of threonine 256 and serine 422 is required for insulin-stimulated activation of SGK-1. Phosphorylated threonine 422 plays an important role in modulating GLUT1 function and regulating hypertension that is associated with hyperinsulinemia, obesity, and insulin resistance.

**Reactivity:** Human SGK1. Mouse and rat SGK1 (92% homologous) have not been tested but are expected to react.

**Applications:** The antibody has been used for Western blotting applications.

**Suggested Working Dilutions:** For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application.

**Storage:** Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

**Expiration Date:** Expires one year from date of receipt when stored as instructed.

**Positive Controls Used:** Active GST tagged-SGK1 protein (Invitrogen, Cat. # PV3818).



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**Related Products:**

**Antibodies:**

SGK1 [pT$^{256}$], Cat. # 44-1260G
IR/IGF1R [pY$^{1158}$], Cat. # 44-802G
IRS-1 [pY$^{612}$], Cat. # 44-816G
AS160 [pT$^{642}$], Cat. # 44-1071G
mTOR [pT$^{2448}$], Cat. # 44-1125G
GSK3β [pS$^{9}$], Cat. # 44-600G
PTEN [pSpTpS$^{380/382/385}$], Cat. # 44-1066G
p70S6 Kinase [pT$^{229}$], Cat. # 44-918G
p70S6 Kinase [pT$^{389}$], Cat .# 44-920G
FOXO1 [pT$^{24}$], Cat. # 44-1240G
FOXO3/1 [pS$^{212}$]/[pS$^{207}$], Cat. # 44-1230G
IR/IGF1R [pY$^{1158}$], Alexa Fluor 488 Conjugate, Cat. # 44-802A1
PTEN [pSpTpS$^{380/382/385}$], Alexa Fluor 488 Conjugate, Cat. # 44-1066A1

**References:**


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Ma, Y.L., et al. (2006) SGK protein kinase facilitates the expression of long-term potentiation in hippocampal neurons. Learn. Mem. 13(2):114-118.

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Boini, K.M., et al. (2006) Serum- and glucocorticoid-inducible kinase 1 mediates salt sensitivity of glucose tolerance. Diabetes 55(7):2059-2066

BelAiba, R.S., et al. (2006) The serum- and glucocorticoid-inducible kinase Sgk-1 is involved in pulmonary vascular remodeling: role in redox-sensitive regulation of tissue factor by thrombin. Circ. Res. 98(6):828-836.

Palmada, M., et al. (2006) SGK1 kinase upregulates GLUT1 activity and plasma membrane expression. Diabetes 55(2):421-427.

Chung, E.J., et al. (2002) Gene expression profile analysis in human hepatocellular carcinoma by cDNA microarray. Mol. Cells 14(3):382-387.

Perrotti, N., et al. (2001) Activation of serum- and glucocorticoid-induced protein kinase (Sgk) by cyclic AMP and insulin. J. Biol. Chem. 276(12):9406-9412.





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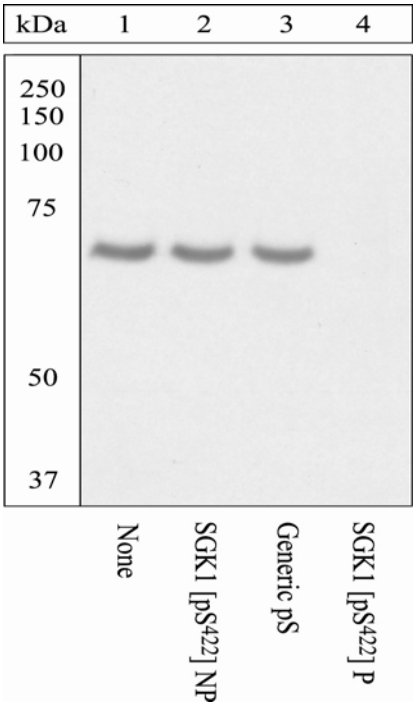


**Antibody Specificity**

Samples (NIH3T3 fibroblast lysates, spiked with 100 ng active GST tagged-SGK1 protein [Invitrogen, Cat. # PV3818]) were resolved on a 10% polyacrylamide gel and transferred to PVDF. The membrane was blocked with a 3% BSA-TBST buffer for one hour at room temperature, and then incubated with the SGK1 [pT$^{422}$] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (1), the non-phosphopeptide corresponding to the phosphopeptide immunogen (2) a generic phosphoserine-containing peptide (3) or the phosphopeptide immunogen corresponding to SGK1 [pS$^{422}$] (4). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ reagent.

The data show that the signal was selectively blocked by the phosphopeptide corresponding to SGK1 [pS$^{422}$] indicating that the signal is phosphorylation site-specific.



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**Western Blotting Procedure**

1. Lyse approximately $10^7$ cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with $ddH_2O$ for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for one hour at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

**Cell Lysis Buffer**
**Formulation:**
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM $Na_3VO_4$
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

**Transfer Buffer**
**Formulation:**
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

**Tris Buffered Saline**
**Formulation:**
20 mM Tris-HCl, pH 7.4
0.9% NaCl

**Blocking Buffer**
**Formulation:**
100 mL Tris buffered saline
3 gm Ig-free BSA
0.1 mL Tween 20



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