

Certificate of Analysis

Product: Protein A Purified Anti-Human SGK-1 [Rabbit]

Code: 200-401-844

Lot #: 14696

Size: 500 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 5.0 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

Background: SGK-1 (also called Serine/threonine-protein kinase Sgk1 and Serum/glucocorticoid-regulated kinase 1) is a protein kinase that plays an important role in cellular stress response. SGK1 activates certain potassium, sodium, and chloride channels, suggesting an involvement in the regulation of processes such as cell survival, neuronal excitability, and renal sodium excretion. Sustained high levels and activity may contribute to conditions such as hypertension and diabetic nephropathy. This kinase mediates cell survival signals, phosphorylates and negatively regulates pro-apoptotic FOXO3A and phosphorylates NEDD4L, which leads to its inactivation and to the subsequent activation of various channels and transporters such as ENaC, Kv1.3, or EAAT1. SGK1 is localized to the cytoplasm and upon phosphorylation is translocated to the nucleus. The kinase is expressed in most tissues with highest levels in the pancreas, followed by placenta, kidney and lung. Induction occurs upon exposure to glucocorticoids and by excessive extracellular glucose or TGF-beta, in cultured cells. SGK-1 is regulated by phosphorylation. Phosphoinositide 3-kinase (PI3-kinase) pathway promotes phosphorylation at Ser-422 which in turn increases the phosphorylation of Thr-256 by PDPK1. The kinase is ubiquitinated by NEDD4L; which promotes proteasomal degradation.

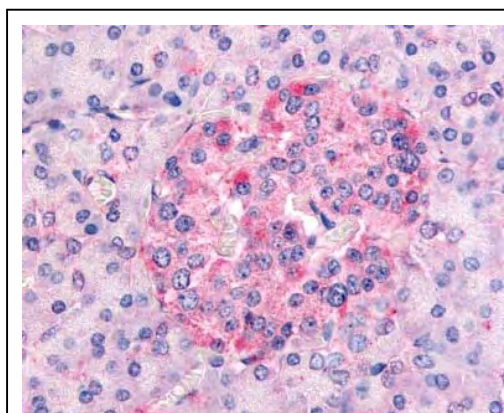


Figure 2. Immunohistochemistry. Rockland's Affinity Purified anti-SGK-1 antibody was used at a 15 µg/ml to detect nuclear and cytoplasmic signal in a variety of tissues including adrenal, heart, liver, ovary, pancreas, placenta, skin, spleen, testes, thyroid and uterus. Low to moderate levels of background staining were noted. This image shows SGK-1 staining of human pancreas. Tissue was formalin-fixed and paraffin embedded.

Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.

Application Note(s): This Protein A purified antibody has been tested for use in ELISA, immunohistochemistry and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band at ~49 kDa in size corresponding to SGK-1 by western blotting in the appropriate cell lysate or extract.

Recommended Dilutions:	ELISA	1:4,000 - 1:16,000
	WESTERN BLOT	1:500 - 1:3,000
	IMMUNOHISTOCHEMISTRY	10 µg/ml to 20 µg/ml
	OTHER APPLICATIONS	User Optimized

Relevant Link(s): Swis-Prot [O00141](#)

NCBI Link [O00141](#)

Purity and Specificity: This affinity purified antibody is directed against human SGK-1 protein. The product was protein A purified from monospecific antiserum. A BLAST analysis was used to suggest reactivity with this protein from human, macaque and chimpanzee sources based on 100% homology for the immunogen sequence. This antibody is expected to cross react with SGK-1 homologues from mouse, rat, dog, chicken, bovine, rabbit and spiny dogfish based on an 92% sequence homology for the immunogen (12/13) from these sources. Cross reactivity with SGK-1 homologues from other sources has not been determined.

Protein Sequence: Human SGK-1, 431 aa, predicted MW 48.9 kDa

1	mtvkteaakg	tltyrsmrgm	vailiafmkq	rrmglnfdiq	kiannsyack	hpevqsilki
61	sqppqpelmn	anpspppsps	qqinlgpssn	phakpsdfhf	lkvigkgsfg	kvllarhkae
121	evfyavkvlq	kkaillkkkee	khimsernvl	lknvkhpfvl	glhfsfqtd	klyfvldyin
181	ggelfyhlqr	ercfleprar	fyaaeiasal	gyhslnivy	rdlkpenill	dsqghivltd
241	fglckenieh	nststfcgt	peylapevlh	kqpydrtdvw	wclgavlyem	lyglppfysr
301	ntaemydni	nkplqlkpni	tnsarhlleg	llqkdrtrkl	gakddfmeik	shvffslinw
361	ddlinkkitp	pfnpnvsqpn	dlrhfdpeft	eepvpnsigk	spdsvlvtas	vkeaaeafllg
421	fsyapptdsf	I				

Immunogen: This Protein A purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 419-431 of human SGK -1.

General References:

Waldegger,S., Barth,P., Raber,G. and Lang,F. (1997) Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. *Proc. Natl. Acad. Sci. U.S.A.* **94** (9), 4440-4445.

Waldegger,S., Erdel,M., Nagl,U.O., Barth,P., Raber,G., Steuer,S., Utermann,G., Paulmichl,M. and Lang,F. (1998) Genomic organization and chromosomal localization of the human SGK protein kinase gene. *Genomics* **51** (2), 299-302.

Kobayashi,T., Deak,M., Morrice,N. and Cohen,P. (1999) Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. *Biochem. J.* **344** PT 1, 189-197.

Kobayashi,T. and Cohen,P. TITLE Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PKD1) and PKD2. *Biochem. J.* **339** (PT 2), 319-328.

Lang,F., Klingel,K., Wagner,C.A., Stegen,C., Warntges,S., Friedrich,B., Lanzendorfer,M., Melzig,J., Moschen,I., Steuer,S., Waldegger,S., Sauter,M., Paulmichl,M., Gerke,V., Risler,T., Gamba,G., Capasso,G., Kandolf,R., Hebert,S.C., Massry,S.G. and Broer,S. (2000) Deranged transcriptional regulation of cell-volume-sensitive kinase hSGK in diabetic nephropathy. *Proc. Natl. Acad. Sci. U.S.A.* **97** (14), 8157-8162.

Brunet,A., Park,J., Tran,H., Hu,L.S., Hemmings,B.A. and Greenberg,M.E. (2001) Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHL1 (FOXO3a). *Mol. Cell. Biol.* **21** (3), 952-965.

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#611-132-122	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10	
#B501-0500	500 g	BLOTTO
#BSA-30	500 ml	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer)
#B304	10 ml	NORMAL GOAT SERUM (NGS)
#KIA-003	MaxTag™ Anti-RABBIT IgG Kit for Immunoblotting	
#MB-070	Blocking Buffer for Fluorescent Western Blotting	

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