ROCKLAND
Immunochemicals for Research
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Certificate of Analysis

Product: Affinity Purified Anti-GSK3 (alpha) pS21 [Rabbit]

Code: 600-401-429

Lot #: 21418

Size: 100 μg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 0.97 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: Glycogen synthase kinase 3 alpha (GSK3A) belongs to the ser/thr family of protein kinases, Cdc2/cdkx subfamily; gsk-3 subsubfamily. It is implicated in the hormonal control of several regulatory proteins including glycogen synthase, myb, and the transcription factor c-jun. GSK3A is a proline-directed serine-threonine kinase that was initially identified as a phosphorylating and inactivating glycogen synthase. Two isoforms, alpha (GSK3A) and beta (GSK3B), show a high degree of amino acid homology. GSK3B is involved in energy metabolism, neuronal cell development, and body pattern formation.

Application Note(s): This affinity-purified antibody has been tested for use in ELISA and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 52 kDa in size corresponding to GSK3A by western blotting in the appropriate cell lysate or extract. Less than 1% reactivity is observed against the non-phosphorylated form of the immunizing peptide. This antibody is phospho specific for pS21 of GSK3A.

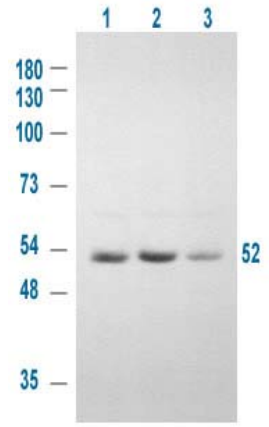

Figure 1. Western blot using Rockland's Affinity Purified anti-GSK3A antibody shows detection of a 52 kDa band corresponding to human GSK3A in various human derived 293T cell extracts. Cells were serum starved for 24 h. Lane 1: control, Lane 2: treated with IGF-1 (100 ng/ml) for 20 min, Lane 3: pre-treated with 10 uM LY294002 (selective PI3K inhibitor) and treated with IGF-1 (100 ng/ml) for 20 min. Lane 1 shows some baseline GSK3A reactivity that is intensified upon stimulation (lane2) and diminished when an inhibitor is added (lane 3). Approximately 20 μg of lysate was run on a SDS-PAGE and transferred onto nitrocellulose followed by reaction with a 1:1,000 dilution of anti-GSK3A antibody. Signal was detected using standard techniques. Personal communication, *Angela Carter, Experimental Therapeutics, Ontario Cancer Inst, Toronto, Canada*

Recommended Dilutions:

ELISA	1:2,000 - 1:10,000
WESTERN BLOT	1:500 - 1:3,000
IF MICROSCOPY	User Optimized
OTHER APPLICATIONS	User Optimized

Purity and Specificity: This affinity-purified antibody is directed against the phosphorylated form of human GSK3A at the pS 21 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross-adsorbed against the non-phosphorylated form of the immunizing peptide. This phospho specific polyclonal antibody reacts with phosphorylated pS21 of human GSK3A. Reactivity with non-phosphorylated human GSK3A is minimal. A BLAST analysis was used to suggest reactivity with this protein from human, chimpanzee and rat based on 100% homology for the immunogen sequence. Cross reactivity with GSK3A homologues from other sources has not been determined.

Relevant Link(s): Swiss Prot: P49840 NCBI Link NP_063937

Protein Sequence: Human GSK3 alpha, 483 aa, predicted MW 51.0 kDa

1	msgggpsggg	pggsg**rarts**	**s**f**aep**ggggg	gggggpggsa	sgpggtgggk	asvgamgggv
61	gasssgggpg	gsgggggsggp	gagtsfpppg	vklgrdsgkv	ttvvatlgqg	persqevayt
121	dikvigngsf	gvvyqarlae	trelvaikkv	lqdkrfknre	lqimrkldhc	nivrlryffy
181	ssgekkdely	lnlvleyvpe	tvyrvarhft	kakltipily	vkvymyqlfr	slayihsqgv
241	chrdikpqnl	lvdpdtavlk	lcdfgsakql	vrgepnvsyi	csryyrapel	ifgatdytss
301	idvwsagcvl	aelllgqpif	pgdsgvdqlv	eiikvlgtpt	reqiremnpn	ytefkfpqik
361	ahpwtkvfks	rtppeaialc	sslleytpss	rlspleacah	sffdelrclg	tqlpnnrplp
421	plfnfsagel	siqpslnail	ipphlrspag	tttltpssqa	ltetptssdw	qstdatptlt
481	nss					

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding aa 16-25 of human GSK3 alpha.

General References:

Gaster,M., et al. (2004) The primary defect in glycogen synthase activity is not based on increased glycogen synthase kinase-3alpha activity in diabetic myotubes. *Biochem. Biophys. Res. Commun*. **319** (4), 1235-1240.

Barry,F.A., Graham,G.J., Fry,M.J. and Gibbins,J.M. (2003) Regulation of glycogen synthase kinase 3 in human platelets: a possible role in platelet function?. *FEBS Lett*. **553** (1-2), 173-178.

Nikoulina,S.E., Ciaraldi,T.P., Mudaliar,S., Carter,L., Johnson,K. and Henry,R.R. (2002) Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. *Diabetes* **51** (7), 2190-2198.

He,X., Saint-Jeannet,J.P., Woodgett,J.R., Varmus,H.E. and Dawid,I.B. (1995) Glycogen synthase kinase-3 and dorsoventral patterning in Xenopus embryos. *Nature* **374** (6523), 617-622.

Related Products:

#600-401-429		Affinity Purified Anti-GSK3 alpha pS21 (Rabbit)
#600-401-444		Affinity Purified Anti-GSK3 alpha (Rabbit)
#611-703-127		Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#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		***Max*Tag**™ Anti-RABBIT IgG Kit for Immunoblotting
#MB-070		Blocking Buffer for Fluorescent Western Blotting