

Certificate of Analysis

Product: Affinity Purified Anti-GSK3B pS9 (Rabbit)

Code: 600-401-424

Lot #: 12474

Size: 100 µg

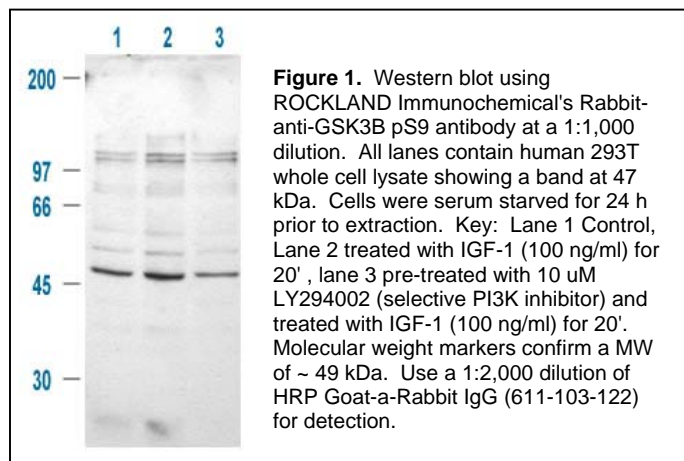
Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.0 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



Background Information: Glycogen synthase kinase-3 (GSK 3) is a proline-directed serine-threonine kinase that was initially identified as a phosphorylating and inactivating glycogen synthase. Two isoforms, alpha (GSK 3A) and beta, show a high degree of amino acid homology. GSK 3B is involved in energy metabolism, neuronal cell development, and body pattern formation. GSK 3B participates in the Wnt signaling pathway and has been implicated in the hormonal control of several regulatory proteins including glycogen synthase, MYB and the transcription factor JUN. GSK 3B phosphorylates JUN at sites proximal to its DNA-binding domain, thereby reducing its affinity for DNA.

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.

Application Note(s): This antibody has been tested for use in ELISA, immunohistochemistry, western blotting and immunoprecipitation. Reactivity in other immunoassays is unknown. Serum starved 293T whole cell lysate is suitable for use as a positive control. Anti-GSK 3B shows a strong signal to GSK 3B in westerns at the estimated molecular weight of 47 kD. This antibody may show very weak reactivity against GSK 3A.

Recommended Dilutions:

ELISA	1:10,000 - 1:50,000
WESTERN BLOT	1:500 - 1:3,000
IMMUNOHISTOCHEMISTRY	1:200 - 1:1,000
IF MICROSCOPY	1:200 - 1:1,000

Purity and Specificity: This affinity purified antibody is directed against the phosphorylated form of human GSK3B at the pS9 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 pS9 of human GSK3B. Reactivity with non-phosphorylated human GSK3B is minimal. A BLAST analysis was used to suggest reactivity with this protein from human, rat, frog, chicken, dog and zebrafish based on 100% homology for the immunogen sequence. Cross reactivity with GSK3B homologues from other sources has not been determined.

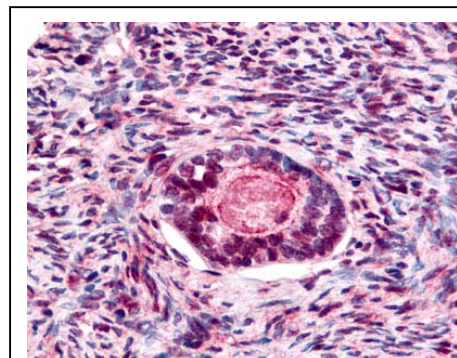


Figure 2. Immunohistochemistry. Rabbit anti-GSK3B pS9 was used at a 1:200 dilution to detect GSK3B by immunohistochemistry in human ovarian cancer tumor tissue. Tissue was formalin-fixed and paraffin embedded. Personal Communication, Alan Yen, [LifeSpanBiosciences](http://LifeSpanBiosciences.com), Seattle, WA.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to the aa 4-12 of human GSK3 beta.

Protein sequence: Human GSK3 beta protein is composed of 420 amino acids (46.7 kDa predicted).

1	MSGRPRTTSF	AESCKPVQQP	SAFGSMKVSR	DKDGSKVTTV	VATPGQGPDR	PQEVSYTDTK
61	VINGSGFGVV	YQAKLCDSGE	LVAIKKVLQD	KRFKNRELQI	MRKLDHCNIV	RLRYFFYSSG
121	EKKDEVYLN	VDYVPETVY	RVARHYSRAK	QTLPIYVKL	YMYQLFRSLA	YIHSFGICHR
181	DIKPQNLLLD	PDTAVLKLCD	FGSAKQLVRG	EPNVSYICSR	YYRAPELIFG	ATDYTSSIDV
241	WSAGCVLAEL	LLGQPIFPD	SGVDQLVEII	KVLGTPTREQ	IREMNPNYTE	FKFPQIKAHP
301	WTKVFRPRTP	PEAIALCSRL	LEYTPARLT	PLEACAHSFF	DELRDPNVKL	PNGRDTPALF
361	NFTTQELSSN	PPLATILIPP	HARIQAAAST	PTNATAASDA	NTGDRGQTNN	AASASASNST

Relevant Links:

GenBank	NM_002093 (Human)	GeneCard	GC03M120822 (Human)
Locus Link	2932 (Human)	Omim	605004 (Human)
SwissProt	P49841 (Human)	Unigene	282359 (Human)

Selected References:

- Stambolic V. and Woodgett J.R. (1994) Mitogen inactivation of glycogen synthase kinase-3 beta in intact cells via serine 9 phosphorylation. *Biochem. J.* **303**:701-704.
- Rhoads A.R. et al. (1999) Radiation hybrid mapping of genes in the lithium-sensitive wnt signaling pathway. *Mol. Psychiatry* **4**:437-442.
- Lau K.F. et al. (1999) Molecular cloning and characterization of the human glycogen synthase kinase-3beta promoter. *Genomics* **60**:121-128.
- Delcomenne M. et al. (1998) Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. *Proc. Natl. Acad. Sci. U.S.A.* **95**:11211-11216.
- Hong Y.-R., et al (2000) Cloning and characterization of a novel human ninein protein that interacts with the glycogen synthase kinase 3beta. *Biochim. Biophys. Acta* **1492**:513-516.
- Dajani R. et al. (2001) Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell* **105**:721-732.
- Bax B. et al. (2001) The structure of phosphorylated GSK-3beta complexed with a peptide, FRATtide, that inhibits beta-catenin phosphorylation. *Structure* **9**:1143-1152.

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.