Anti-Phospho-GSK3 beta (S9) Antibody [SY02-71] - BSA and Azide free

HA750128

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 47 kDa
Clone number:	SY02-71

Description: Glycogen synthase kinase-3α (GSK-3α) and GSK-3β are highly similar isoforms of serine/threonine kinases that regulate metabolic enzymes and transcription factors, which are responsible for coordinating processes such as glycogen synthesis and cell adhesion. GSK-3β activity is also required for nuclear activity of Rel dimers, which mediate an anti-apoptotic response to TNFα in mice. GSK-3 catalytic kinase activity is controlled through differential phosphorylation of serine/threonine residues, which have an inhibitory effect, and tyrosine residues, which have an activating effect. Growth factor stimulation of mammalian cells expressing GSK-3α and GSK-3β induces phosphorylation of Ser 21 and Ser 9, respectively, through a phosphatidylinositol 3-kinase (PI 3-K)-protein kinase B (PKB)-dependent pathway, thereby enhancing proliferative signals. Additionally, GSK-3 physically associates with cAMP-dependent protein kinase A (PKA), which phosphorylates Ser 21 of GSK-3α or Ser 9 of GSK-3β and inactivates both forms. GSK-3α/β is positively regulated by phosphorylation on Tyr 279 and Tyr 216, respectively. Activated GSK-3α/β participates in energy metabolism, neuronal cell development, and body pattern formation. Tyrosine dephosphorylation of GSK-3 is involved in its extracellular signal-dependent inactivation.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser9 of Human GSK3 beta aa 1-50 / 420.

Positive control: HeLa treated with 100nM Calyculin A for 30 minutes cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 100ng/mL PDGF for 5 minutes cell lysate, C6 cell lysate, C6 treated with 100ng/mL PDGF for 5 minutes cell lysate, C6 treated with 150nM insulin for 15 minutes cell lysate, HeLa cells treated with 100nM Calyculin A for 30 minutes, NIH/3T3 cells treated with 100ng/mL PDGF for 5 minutes, C6 cells treated with 100ng/mL PDGF for 5 minutes, human colon carcinoma tissue, human breast tissue, human breast carcinoma tissue, human kidney tissue, human pancreas tissue.

Subcellular location: Cytoplasm, Nucleus, Cell membrane.

Database links: SwissProt: P49841 Human | Q9WV60 Mouse | P18266 Rat

Recommended Dilutions:

WB	1:5,000-1:10,000
IF-Cell	1:100-1:500
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200
FC	1:1,000

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

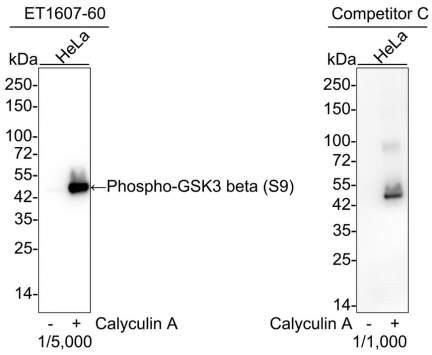

Fig1: Western blot analysis of Phospho-GSK3 beta (S9) on different lysates with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: HeLa treated with 100nM Calyculin A for 30 minutes cell lysate (15 µg/Lane)

Predicted band size: 47 kDa
Observed band size: 47 kDa

Exposure time: Lane 1-2 (left): 46 seconds; ECL: K1801;
Exposure time: Lane 1-2 (right): 45 seconds; ECL: K1802;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750128) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

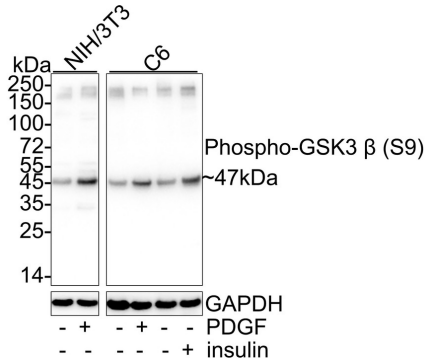

Fig2: Western blot analysis of Phospho-GSK3 beta (S9) on different lysates with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/5,000 dilution.

Lane 1: NIH/3T3 cell lysate (20 µg/Lane)
Lane 2: NIH/3T3 treated with 100ng/mL PDGF for 5 minutes cell lysate (20 µg/Lane)
Lane 3: C6 cell lysate (20 µg/Lane)
Lane 4: C6 treated with 100ng/mL PDGF for 5 minutes cell lysate (20 µg/Lane)
Lane 5: C6 cell lysate (20 µg/Lane)
Lane 6: C6 treated with 150nM insulin for 15 minutes cell lysate (20 µg/Lane)

Predicted band size: 47 kDa
Observed band size: 47 kDa
Exposure time: 6 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750128) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

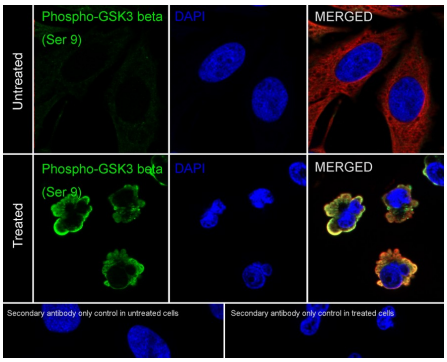

Fig3: Immunocytochemistry analysis of HeLa cells treated with 100nM Calyculin A for 30 minutes labeling Phospho-GSK3 beta (S9) with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

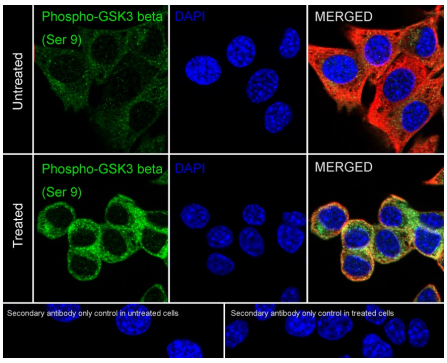

Fig4: Immunocytochemistry analysis of NIH/3T3 cells treated with 100ng/mL PDGF for 5 minutes labeling Phospho-GSK3 beta (S9) with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

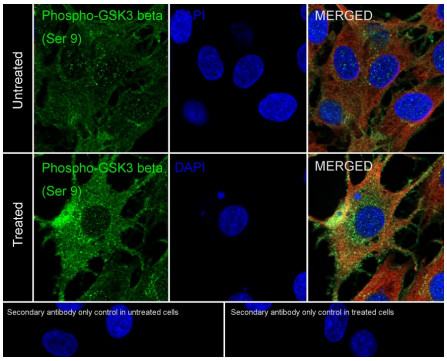

Fig5: Immunocytochemistry analysis of C6 cells treated with 100ng/mL PDGF for 5 minutes labeling Phospho-GSK3 beta (S9) with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

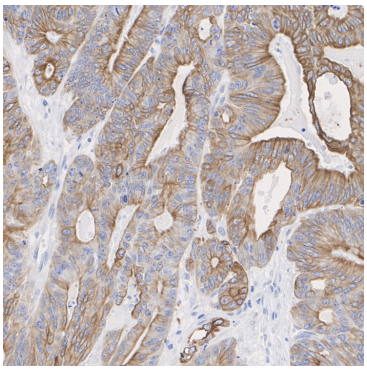

Fig6: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750128) at 1/50 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

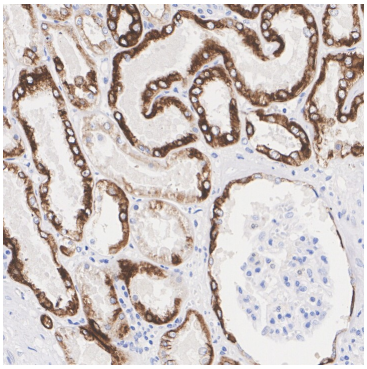

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Phospho-GSK3 beta (S9) antibody (HA750128) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750128) at 1/50 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

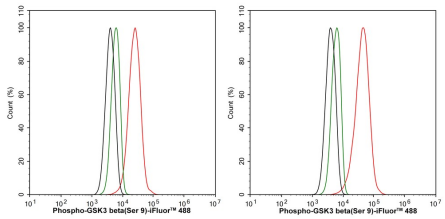

Fig8: Flow cytometric analysis of NIH/3T3 cells treated with 100ng/mL PDGF for 5 minutes labeling Phospho-GSK3 beta (S9).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750128, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Ji WT et al. Areca nut extract induces pyknotic necrosis in serum-starved oral cells via increasing reactive oxygen species and inhibiting GSK3 : an implication for cytopathic effects in betel quid chewers. PLoS One 8:e63295 (2013).
2. Yan X et al. Huaier aqueous extract inhibits ovarian cancer cell motility via the AKT/GSK3 / -catenin pathway. PLoS One 8:e63731 (2013).