

GSK3 Antibody Sampler Kit

1 Kit
 (5 x 40 µl)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
GSK-3α (D80E6) Rabbit mAb	4337	40 µl	51 kDa	Rabbit IgG
Phospho-GSK-3β (Ser9) (5B3) Rabbit mAb	9323	40 µl	46 kDa	Rabbit IgG
Phospho-GSK-3α/β (Ser21/9) (37F11) Rabbit mAb (GSK-3α Preferred)	9327	40 µl	51 kDa α; 46 kDa β	Rabbit IgG
GSK-3β (27C10) Rabbit mAb	9315	40 µl	46 kDa	Rabbit IgG
Phospho-GSK-3α (Ser21) (36E9) Rabbit mAb	9316	40 µl	51 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The GSK3 Antibody Sampler Kit contains primary and secondary antibodies to perform four Western mini-blot with each antibody.

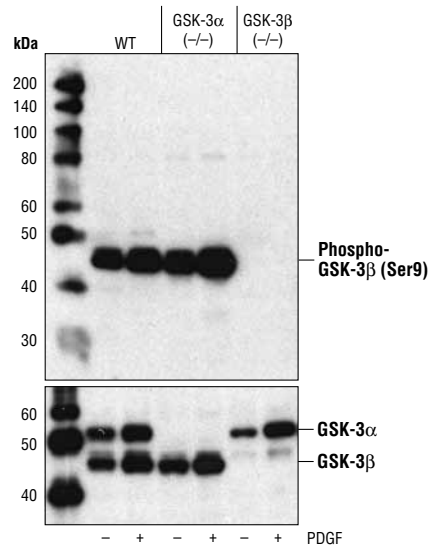
Background: Glycogen synthase kinase-3 (GSK-3) was initially identified as an enzyme that regulates glycogen synthesis in response to insulin (1). GSK-3 is an ubiquitously expressed serine/threonine protein kinase that phosphorylates and inactivates glycogen synthase. GSK-3 is a critical downstream element of the PI3 kinase/Akt cell survival pathway, and its activity can be inhibited by Akt-mediated phosphorylation at Ser21 of GSK-3α and Ser9 of GSK-3β (2,3). GSK-3 has been implicated in the regulation of cell fate in *Dictyostelium*, and is a component of the Wnt signaling pathway required for *Drosophila*, *Xenopus* and mammalian development (4). GSK-3 has been shown to regulate cyclin D1 proteolysis and subcellular localization (5).

Specificity/Sensitivity: Each antibody in the GSK3 Antibody Sampler Kit recognizes only its specific target and does not cross-react with other family members.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues around Ser9 of human GSK-3β, residues around Ser21 of human GSK-3α, the N terminal sequence of human GSK-3β and to the sequence of human GSK-3α protein.

Background References:

- Welsh, G.I. et al. (1996) *Trends Cell. Biol.* 6, 274–279.
- Srivastava, A.K. and Pandey, S.K. (1998) *Mol. Cell. Biochem.* 182, 135–141.
- Cross, D.A. et al. (1995) *Nature* 378, 785–789.
- Nusse, R. (1997) *Cell* 89, 321–323.
- Diehl, J.A. et al. (1998) *Genes Dev.* 12, 3499–3511.



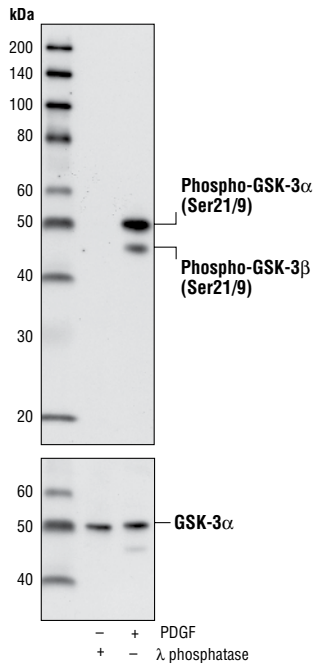
Western blot analysis of extracts from wild type (lanes 1,2), GSK-3α (-/-) (lanes 3,4) or GSK-3β (-/-) (lanes 5,6) mouse embryonic fibroblast cells (MEFs), untreated or PDGF treated, using **Phospho-GSK-3β (Ser9) (5B3) Rabbit mAb #9323** (upper) and **GSK-3α/β Antibody** (lower). (MEF wild type, GSK-3α (-/-) and GSK-3β (-/-) cells were kindly provided by Dr. Jim Woodgett, University of Toronto, Canada).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

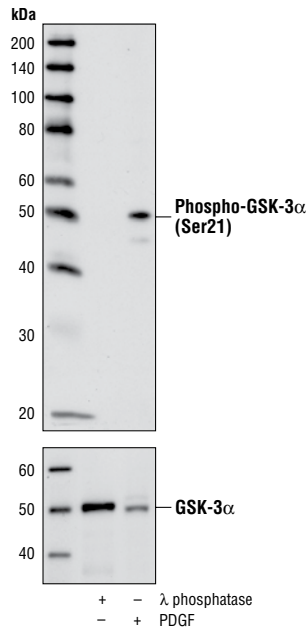
Recommended Antibody Dilutions:
 Western blotting 1:1000

Please visit www.cellsignal.com for a complete listing of recommended companion products.

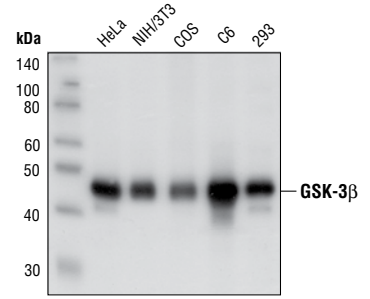
Selected Rabbit monoclonals are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063) from Epitomics, Inc.



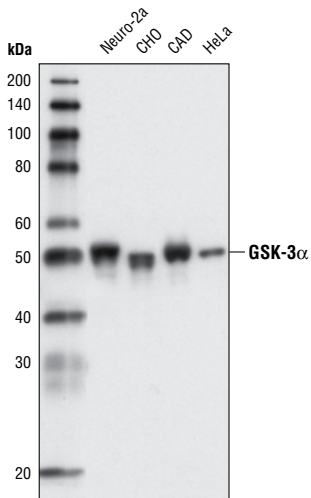
Western blot analysis of extracts from COS-7 cells, λ -phosphatase- or PDGF-treated, using **Phospho-GSK-3 α / β (Ser21/9) (37F11) Rabbit mAb (GSK-3 α Preferred) #9327** (upper) or **GSK-3 α Antibody #9338** (lower).



Western blot analysis of extracts from COS-7 cells, λ -phosphatase or PDGF-treated, using **Phospho-GSK-3 α (Ser21) (36E9) Rabbit mAb #9316** (upper) or **GSK-3 α Antibody #9338** (lower).



Western blot analysis of extracts from various cell lines using **GSK-3 β (27C10) Rabbit mAb #9315**.



Western blot analysis of extracts from various cell types using **GSK-3 α (D80E6) Rabbit mAb #4337**.

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
10. **Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.