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GSK-3α Mouse Monoclonal Antibody (Clone MS-1G7)

Cat# CY-M1031

100 µg (1 mg/ml x 100 µL)

Clone Name	Applications	Species Cross-reactivity	Molecular Wt.	Source Isotype
MS-1G7	WB	H, M, R	51 kDa	Mouse IgG1

Background: The serine/threonine proline-directed kinase, glycogen synthase kinase-3 (GSK-3) plays a central role in a wide variety of normal and pathological cellular processes. First described as the kinase capable of phosphorylating and inactivating glycogen synthase, GSK-3 is now recognized as a key regulator of many normal processes such as cellular development, proliferation, and survival. Dysregulation of GSK-3 activity has been linked to various human diseases including Alzheimer's disease, diabetes, cancer, heart disease, schizophrenia, and mood disorders.

It is generally considered constitutively active and found as two isoforms, α and β (51 and 47 kDa, respectively), that have 97% sequence homology in their catalytic domains, and generally (but not always) have similar biological effects.

GSK-3 can be regulated either by Wnt signaling or by the phosphatidylinositol (PI) 3-kinase/Akt pathways. Wnt signaling leads to an inhibition of the phosphorylation of β-catenin by GSK-3 and a corresponding increase in transcriptional activation of beta-catenin/Tcf target genes (1). Activation of PI3-kinase in response to stimulation of cells by insulin or growth factor causes a decrease in GSK-3 activity because of an inhibitory phosphorylation by Akt of GSK-3β on serine 9 or GSK-3αon serine 21 (2). The resulting inhibition of GSK-3 plays a critical role in signaling cell growth and survival downstream of PI3-kinase. In the absence of growth factor stimulation, GSK-3 is active and can inhibit cell proliferation as well as induce apoptosis (3–6). The substrates of GSK-3 that have been implicated in regulation of cell proliferation and survival include the translation initiation factor eIF2B (7, 8), cyclin D1 (3), the Bcl-2 family member Mcl-1 (9), and a variety of transcription factors that are phosphorylated by GSK-3 either in vitro or in vivo (5, 10).

Specificity/Sensitivity: Human GSK-3α Monoclonal Antibody detects human GSK-3α by western blotting.

Source/Purification: Monoclonal antibody is produced by immunizing mice with a full length of human GSK-3α, expressed in *E.coli.*. IgG is purified by protein A-sepharose chromatography.

Recommended Antibody Dilutions: Western blotting: 1-2 ug/mL.

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western Blotting **IP:**Immunoprecipitation **IHC:**Immunohistochemistry **IC:**Immunocytochemistry **F:**Flow cytometry **E:**ELISA **FP:**Fluorescence Polarization assay
Species Cross-Reactivity Key: H:human **M:**mouse **R:**rat **Hm:**hamster **Mk:**monkey **Mi:**mink **C:**chicken **X:***Xenopus* **Z:**zebra fish **All:**all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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Selected Application References:

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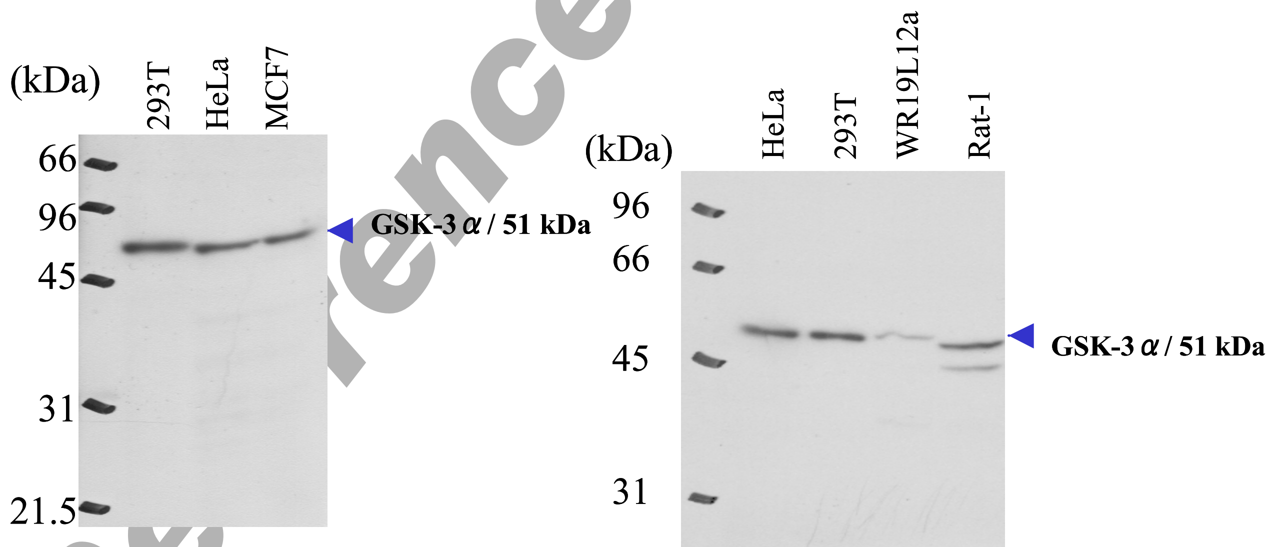
10. Doble, B. W., and Woodgett, J. R. (2003) J. Cell Sci. 116, 1175–1186

Fig.1 Western blot analysis of GSK-3α



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Western Immunoblotting Protocol

Solutions and Reagents

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

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 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
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%).
10X TBS (Tris-buffered saline): 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).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent; 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%).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

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 cm2 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).
8. Electrotransfer to nitrocellulose membrane.

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 3 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 3 times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 ml of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 ml of TBS/T.

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Detection of Proteins

1. Incubate membrane with 4 ml ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten seconds exposure should indicate the proper exposure time.

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