Mouse (monoclonal) Anti-GSK-3α/β Antibody Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number:	44-610
Lot Number:	See product label
Quantity/Volume:	100 µg (1.0 mg/mL)
Form of Antibody:	Purified immunoglobulin in phosphate buffer, pH 7.4.
Preservation:	0.01% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Isotype:	IgG2a
Purification:	Purified by Protein G affinity chromatography.
Immunogen:	The antibody was produced in mouse against a recombinant *Xenopus laevis* protein.
Specificity:	The antibody recognizes two isoforms of glycogen synthase kinase, GSK-3α, a 51 kDa protein and GSK-3β, a 47 kDa protein. Glycogen synthase kinase (GSK) is a protein serine kinase involved in the control of regulatory proteins such as glycogen synthase and the transcription factor, c-jun. In addition, it has been associated with the regulation of the microtubule-binding protein, Tau, thus indicating a potential role in the pathogenesis of Alzheimer's disease.
Species Reactivity:	This antibody recognizes human, mouse, rat and *Xenopus laevis* GSK-3α/β.
SWISS-PROT and TrEMBL Accession Numbers:	GSK-3α: P49840 GSK-3β: P49841
Applications:	The antibody has been used in Western blotting, ELISA, and immunoprecipitation.
Suggested Working Dilutions:	For Western blot and ELISA, we recommend using the antibody at a concentration of 0.5-1.0 µg/mL. For immunoprecipitation, we recommend using 5-10 µg per reaction. The optimal antibody concentration should be determined for each specific application.
Recommended Positive Controls:	PC-12, A431 and 3T3-L1 cells
Storage:	Store at –20°C. Upon initial thawing, apportion into working aliquots and store at –20°C. Avoid repeated freeze-thaw cycles to prevent denaturing the antibody.
Expiration Date:	Expires one year from date of receipt when stored as instructed.
Related Products:	GSK-3β [pS^{9}] Phosphospecific Antibody, Cat. # 44-600G GSK-3α/β [$pY^{216/279}$] Phosphospecific Antibody, Cat. # 44-604G

This product is for research use only. Not for use in diagnostic procedures.

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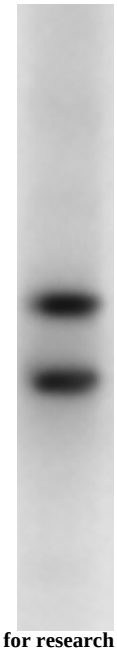

Western Blot Analysis
Proteins were resolved from MCF-7 cell extracts by SDS-PAGE. The proteins were transferred to PVDF membrane and incubated with this mouse monoclonal anti-GSK-3α/β antibody at 0.5 µg/mL. The signal was detected using a goat $F(ab')_2$ anti-mouse IgG Alkaline Phosphatase conjugated antibody (cat. # AMI4405) at a 1:5000 dilution and the membrane was incubated with CDP-substrate using the WesternStar™ method (Tropix). The membrane was then exposed to Kodak BioMax film.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (BioSource catalog number FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Nitrocellulose is also suitable.
7. Soak the PVDF membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a concentration of 0.1-1.0 µg/mL in Tris buffered saline supplemented with 1% BSA and 0.1% Tween 20 for 2 hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-mouse IgG alkaline phosphatase conjugate (AMI4405) or goat $F(ab')_2$ anti-mouse IgG horseradish peroxidase conjugate (AMI4405) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma catalog number P2714 may be used)

Transfer Buffer Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer Formulation:
100 mL Tris buffered saline
4 gm BSA
0.1 mL Tween 20

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