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Polyclonal Antibody to Glycogen Synthase Kinase 3 alpha/beta (GSK3a/b) - Purified

Alternate names: EC 2.7.11.26, EC=2.7.11.26, GSK-3 alpha, GSK-3 beta, GSK3A, GSK3B, Glycogen synthase kinase-3 alpha, Glycogen synthase kinase-3 beta

Catalog No.: BM7002

Quantity: 0.1 mg

Concentration: 1.0 mg/ml

Background: 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.

Host / Isotype: Mouse / IgG2a

Immunogen: Recombinant Xenopus laevis protein.

Format:
State: Liquid Ig fraction
Purification: Protein G chromatography
Buffer System: Phosphate buffer, pH 7.4., 0.01 % sodium azide as preservative

Applications:
Western blot (0.5-1.0 µg/mL).
ELISA (0.5-1.0 µg/mL).
Immunoprecipitaion (5-10 µg per reaction).
Use PC-12, A431 and 3T3-L1 cells as positive control.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
The antibody recognizes two isoforms of glycogen synthase kinase, GSK-3α, a 51 kDa protein and GSK-3β, a 47 kDa protein.
Species: Human, mouse, rat and Xenopus laevis.
Other species not tested.

Storage:
Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:
Vary, T.C., et al. (2002) Phosphorylation of eukaryotic initiation factor eIF2B epsilon in skeletal muscle during sepsis. Am. J. Physiol.- Endocrinology and Metabolism 283(5):E1032-E1039.
Demarchi, F., et al. (2001) Gas 6 anti-apoptotic signaling requires NF-kappaB activation. J. Biol. Chem 276(34):31738-31744.
Kitaura, J., et al. (2000) Akt-dependent cytokine production in mast cells. J. Exp. Med. 192(5):729-739.
Summers, S., et al. (1999) The role of glycogen synthase kinase 3β in insulin-stimulated glucose metabolism. J. Biol. Chem. 274:17,934-17,940.

Protocols: Western Blotting Procedure

1. Lyse approximately 10e7 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. 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 ultracentrifugedat 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 ddH2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the 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) for one hour at room temperature or 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-rabbit IgG alkaline phosphatase conjugate or goat F(ab)2 anti-rabbit IgG horseradish peroxidase conjugate 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 Na4P2O7
2 mM Na3VO4
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 Cat. # 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
5 gm BSA
0.1 mL Tween 20

Pictures:

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 at a 1:5000 dilution and the membrane was incubated with CDP-substrate using the WesternStarTM method (Tropix). The membrane was then exposed to Kodak BioMax film.