Technical Data Sheet

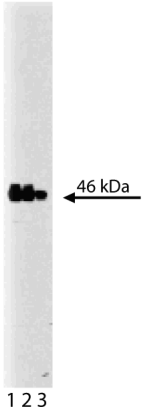
Purified Mouse Anti-GSK-3β

Product Information

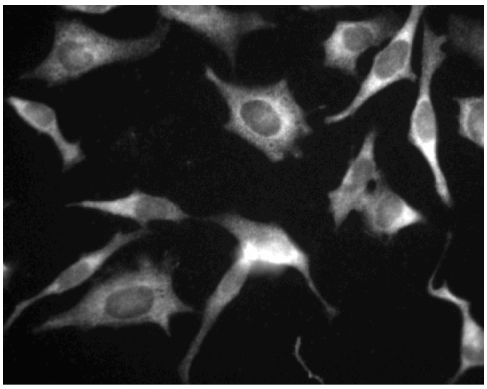
Material Number:	**610202**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	7/GSK-3b
Immunogen:	Rat GSK-3β aa. 1-160
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Mouse Tested in Development: Chicken, Dog, Human, Rat
Target MW:	46 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Glycogen Synthase Kinase-3β (GSK-3β) is a serine/threonine kinase that affects glycogen metabolism by phosphorylating and, thus, down-regulating the activity of muscle glycogen synthase. GSK-3β is identical to the Tau Protein Kinase 1 (TPK 1) which plays a role in the formation of the histopathological lesions of Alzheimer's disease (AD). In AD, many neurons contain intracytoplasmic neurofibrillary tangles (NFT) that are composed of ubiquitin and highly phosphorylated tau proteins, members of a family of microtubule-assocaited phosphoproteins. Phosphorylation of the tau proteins by GSK-3β converts them into paired helical filaments (PHF) which are found in NFT and degenerative neurites of senile plaques. This ability of GSK-3β to convert tau proteins to PHF has been demonstrated in vitro using primary cultures of embryonic rat hippocampal neurons.



Western blot analysis of GSK-3β on a RSV-3T3 lysate. *Lane 1: 1:2500, lane 2: 1:5000, lane 3: 1:10000 dilution of the anti-GSK-3β antibody.*



Immunofluorescent staining of HeLa (ATCC CCL-2) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-GSK-3β antibody. The second step reagent was Alexa Fluor® 555 goat anti-mouse IgG (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) and cells and worked with both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Immunoprecipitation	Reported
Immunohistochemistry	Reported

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.
   OR
   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritifed_reagents.jsp
Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

Bijur GN, Jope RS. Proapoptotic stimuli induce nuclear accumulation of glycogen synthase kinase-3 beta. *J Biol Chem.* 2001; 276(40):37436-37442. (Clone-specific: Flow cytometry, Immunofluorescence, Immunoprecipitation, Western blot)

Etienne-Manneville S, Hall A. Cdc42 regulates GSK-3beta and adenomatous polyposis coli to control cell polarity. *Nature.* 2003; 421(6924):753-756. (Clone-specific: Immunofluorescence, Immunoprecipitation, Western blot)

Lucas JJ, Hernandez F, Gomez-Ramos P, Moran MA, Hen R, Avila J. Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. *EMBO J.* 2001; 20(1-2):27-39. (Clone-specific: Immunohistochemistry)

Morisco C, Seta K, Hardt SE, Lee Y, Vatner SF, Sadoshima J. Glycogen synthase kinase 3beta regulates GATA4 in cardiac myocytes. *J Biol Chem.* 2001; 276(30):28586-28597. (Clone-specific: Gel shift, Immunofluorescence, Immunoprecipitation, Western blot)

Takashima A, Noguchi K, Sato K, Hoshino T, Imahori K. Tau protein kinase I is essential for amyloid beta-protein-induced neurotoxicity. *Proc Natl Acad Sci U S A.* 1993; 90(16):7789-7793. (Biology)