

Technical Data Sheet

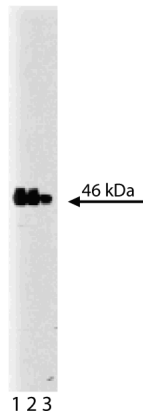
Purified Mouse Anti-GSK-3β**Product Information**

Material Number:	610201
Size:	50 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 Molecular Weight:	46 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

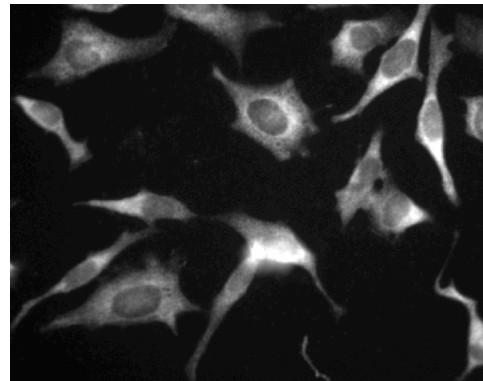
Description

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-associated 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.

This antibody is routinely tested by the Western blot and bioimaging analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



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 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 methanol fix/perm protocol (below) and the anti-GSK-3 antibody. The second step reagent was Alexa Fluor® 555 goat anti-mouse IgG (Invitrogen). The image was taken on a Pathway 850 imager using a 20x objective. This antibody also stained A549 and U2OS and cells and worked with both the Triton X100 and methanol fix/perm protocols (see Recommended Assay Procedure).

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit bdbiosciences.com/how_to_order/

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2006 BD

**BD****BD Biosciences**

Preparation and Storage

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

Store undiluted at -20° C.

Application Notes

Application

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

Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Igs	1.0 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/pharming/en/protocols for technical protocols.
3. 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.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

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)