Technical Data Sheet

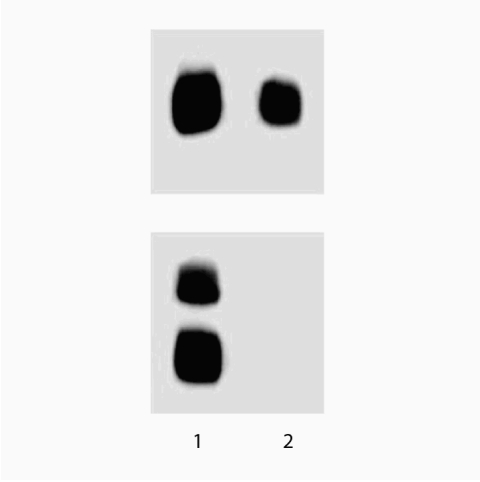
Purified Mouse Anti-GSK-3β (pY216)

Product Information

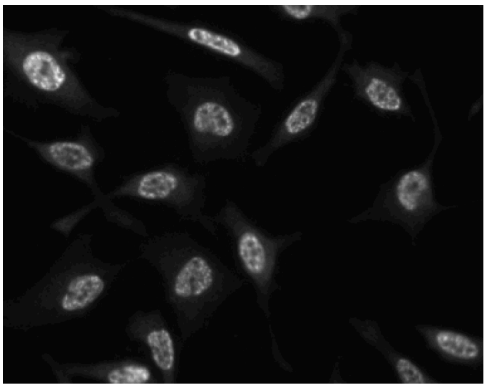
Material Number:	**612312**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	13A
Immunogen:	Rat GSK-3β (pY216) Peptide
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Mouse Tested in Development: Human, Rat
Target MW:	46 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Glycogen Synthase Kinase-3β (GSK-3β) is a serine/threonine kinase that affects glycogen metabolism by phosphorylating and down-regulating the activity of muscle glycogen synthase. GSK-3β is identical to the Tau Protein Kinase I (TPK I) that plays a role in the formation of the histopathological brain lesions of Alzheimer's disease (AD). Phosphorylation of the cytoskeletal protein, tau, by GSK-3β converts these proteins into paired helical filaments (PHF) which are found in the neurofibrillary tangles and degenerative neurites of AD patients. Regulation of GSK-3β activity through both serine and tyrosine phosphoylation is a critical determinant of cell death or survival. Factors that promote cell survival, such as growth factors, activate Akt which, in turn phosphorylates GSK-3β at Ser-9, leading to inactivation of its kinase activity. On the contrary, events that promote cell death, such as growth factor removal, cause increases in phosphorylation within the catalytic domain at Tyr-216 and stimulate kinase activity. Thus GSK-3β is a tightly regulated death promoting kinase that regulates the activity of various proteins, including cytokeletal and enzymatic proteins.



RSV-3T3 lysate was either left untreated (lane 1) or treated (lane 2) with 50 µg/ml alkaline phosphatase for 30 minutes at 37°C. *The top panel was probed with an anti-GSK-3β (Cat. No. 610201) antibody and the bottom was probed with the anti-GSK-3β (pY216) antibody (Cat. No. 612312) at a 1:1000 dilution.*



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 (pY216) antibody. The second step reagent was Alexa Fluor® 555 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) cells and worked with both the Triton™ X-100 and allcohol perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at -20°C.

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

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

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
610201	Purified Mouse Anti-GSK-3β	50 µg	7/GSK-3b
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)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	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/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

Bhat RV, Shanley J, Correll MP, et al. Regulation and localization of tyrosine216 phosphorylation of glycogen synthase kinase-3beta in cellular and animal models of neuronal degeneration. *Proc Natl Acad Sci U S A.* 2000; 97(20):11074-11079. (Biology)

Hartigan JA, Johnson GV. Transient increases in intracellular calcium result in prolonged site-selective increases in Tau phosphorylation through a glycogen synthase kinase 3beta-dependent pathway. *J Biol Chem.* 1999; 274(30):21395-21401. (Biology)