Technical Data Sheet

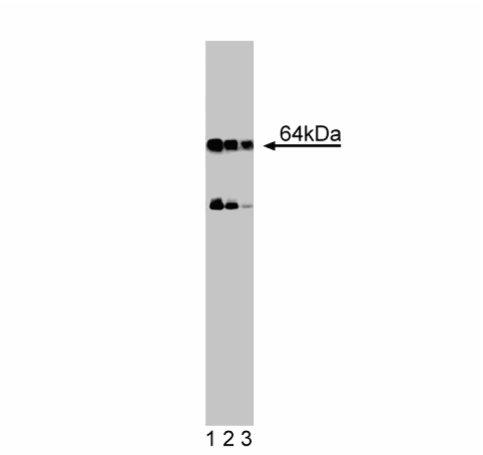
Purified Mouse Anti-Tubby

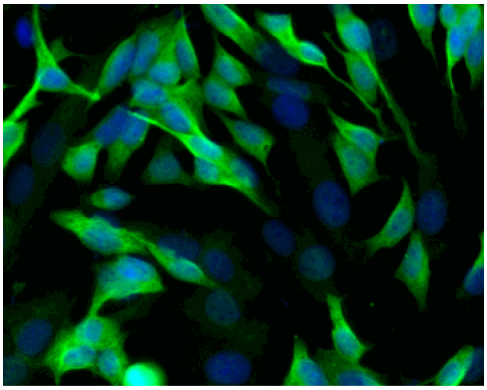
Product Information

Material Number:	**612106**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	40/Tubby
Immunogen:	Mouse Tubby aa. 102-205
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Mouse Tested in Development: Human, Rat
Target MW:	64 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Tubby is an autosomal recessive syndrome characterized by maturity-obesity, insulin resistance, infertility, and cochlear and retinal degeneration. Tubby protein (Tub) is highly expressed in brain, especially hypothalamus where body weight regulation is controlled. The sequence of normal tubby includes putative tyrosine phosphorylation sites for SH-2 protein binding and a nuclear localization signal (NLS), while mutant tubby contains a 24 intron amino-acid insert substituted for 44 C-terminal amino acids. In PC12 cells, insulin induces tyrosine phosphorylation. In vitro, tubby is phosphorylated by insulin receptor kinase, Abl, JAK2, and upon phosphorylation tubby associates with the SH2 domains of Abl, Lck, phospholipase Cγ. The C-terminal region of tubby binds to phosphatidylinositol 4,5-bis-phosphate, which facilitates localization to the plasma membrane. Receptor-mediated activation of Gαq releases tubby from the plasma membrane through the action of phospholipase C-B. This allows translocation of tubby to the nucleus where it may regulate transcription. Thus, tubby is a transcription regulator activated by the G-protein phospholipid signaling pathway.





Western blot analysis of Tubby on mouse cerebrum lysate (left). *Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of Tubby.*

Immunofluorescent staining of SK-N-SH cells (right). *Cells were seeded in a 384 well collagen coated Microplates (Material # 353962) at ~ 8,000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) and the anti-Tubby antibody. The second step reagent was Alexa Fluor® 488 goat anti mouse Ig (Invitrogen). The image was taken on a Pathway 855 or 435 imager using a 20x objective. This antibody also stained SH-SY5Y, C6, U87 and U373 cells using both the Triton X100 and methanol fix/perm protocols (see Recommended Assay Procedure).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20°C.

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/**

Application Notes

Application

Western blot	Routinely Tested
Immunofluorescence	Tested During Development

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
611455	Mouse Cerebrum Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

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. 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

Kapeller R, Moriarty A, Strauss A, et al. Tyrosine phosphorylation of tub and its association with Src homology 2 domain-containing proteins implicate tub in intracellular signaling by insulin. *J Biol Chem.* 1999; 274(35):24980-24986.(Biology)

Kleyn PW, Fan W, Kovats SG, et al. Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family. *Cell.* 1996; 85(2):281-290.(Biology)

Santagata S, Boggon TJ, Baird CL, et al. G-protein signaling through tubby proteins. *Science.* 2001; 292(5524):2041-2050.(Biology)