Technical Data Sheet

Purified Mouse Anti-MAP2B

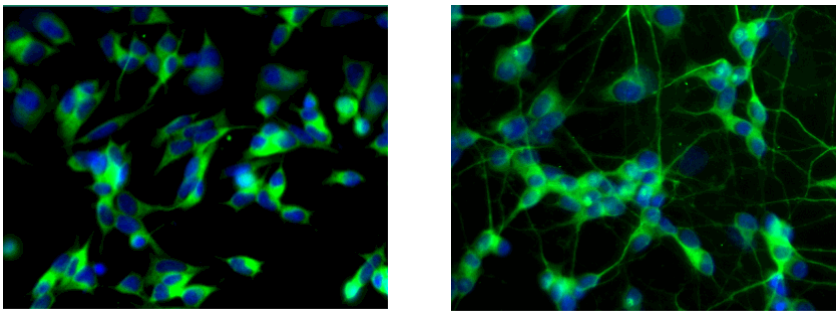
Product Information

Material Number:	**610460**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	18/MAP2B
Immunogen:	Human MAP2B aa. 19-219
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Rat Tested in Development: Mouse, Human
Target MW:	280 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Microtubule-**a**ssociated **p**roteins (MAPs) play a crucial role in the development and structure of nerve cells. These proteins are important for the assembly and stability of microtubules during neurite outgrowth and for the morphology of neuronal processes, such as dendrites, MAP2, specifically localized in dendrites, has four known isoforms produced by alternative splicing of the transcript. These isoforms, MAPs A, B, C, and D, are expressed at various stages of neuronal development. MAP2B is a 280-kDa protein expressed throughout brain development. It is composed of several highly conserved domains that are flanked by domains with extensive sequence divergence. An N-terminal conserved domain overlaps with a binding domain for the regulatory subunit of the cAMP-dependent kinase II, while a C-terminal conserved domain overlaps with a microtubule-binding domain. Secondary structure prediction suggests that the portion of MAP2B extending from the microtubule surface is composed of a number of alpha-helices separated by small turns which may account for the extended, yet flexible, structure of MAP2B.

Western blot analysis of MAP2B on rat brain lysate. *Lane 1: 1:2500, lane 2: 1:5000, lane 3: 1:10000 dilution of MAP2B.*



Immunofluorescent staining of undifferentiated (left) and differentiated (right) SH-SY5Y cells. Cells were seeded in a collagen coated 384 well imaging plate (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-MAP2B antibody. Differentiated cells were seeded in a 96 well, collagen coated imaging plate (Material # 353219) at ~ 5,000 cells per well. Cells were incubated with 50 mM ATRA (Sigma, R2625) for 5 days, followed by 50 ng/ml BDNF (Sigma, B3795) for 5 days. Differentiated cells were fixed and stained using the methanol fix/perm protocol, and the anti-MAP2B antibody. The second step reagent in both cases was Alexa Fluor® 488 goat anti mouse Ig (Invitrogen). The images were taken on a Pathway 855 or 435 imager using a 20x objective. This antibody also stained undifferentiated SK-N-SH 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.

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Application Notes

Application

Western blot	Routinely Tested
Immunofluorescence	Tested During Development
Immunohistochemistry	Tested During Development
Immunoprecipitation	Not Recommended

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.

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

Kanaani J, el-Husseini Ael-D, Aguilera-Moreno A, Diacovo JM, Bredt DS, Baekkeskov S. A combination of three distinct trafficking signals mediates axonal targeting and presynaptic clustering of GAD65. *J Cell Biol.* 2002; 158(7):1229-1238.(Clone-specific: Immunofluorescence)

Kindler S, Schulz B, Goedert M, Garner CC. Molecular structure of microtubule-associated protein 2b and 2c from rat brain. *J Biol Chem.* 1990; 265(32):19679-19684.(Biology)