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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-Synaptophysin mAb

Code No.	Clone	Subclass	Quantity	Concentration
D073-3	171B5	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Synaptophysin (also named as SVP38 and p38) is an acidic Ca^{2+} binding glycoprotein of about 38 kDa which exist largely in synaptic vesicles. It has 4 transmembrane regions and reported that it is a major cholesterol-binding protein in synaptic vesicles. Synaptophysin may regulate synaptic exocytosis by competing with proteins such as SNAP25 and syntaxins for binding synaptobrevin (vesicle-associated membrane protein, or VAMP). It may also participate in synaptic endocytosis, which contributes to rapid recycling of synaptic vesicle.

SOURCE: This antibody was purified from hybridoma (clone 171B5) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell X63-Ag8-653 with Balb/c mouse splenocyte immunized with purified synaptic vesicle fraction from guinea pig cerebrum.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with Synaptophysin on Western blotting and Immunohistochemistry.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; 10 µg/mL
Immunocytochemistry; Not tested*
* It is reported that this antibody can be used in Immunocytochemistry in the reference number 3).
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat*	Other
Tissues	Frontal lobe, Spine	Not tested	Not tested	See ref. 11)
Reactivity on IHC	+			

*It is reported that this antibody reacts with rat hippocampal synaptosomes in Western blotting[1)].

REFERENCES:

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2) Kojima, N., *et al., Neuroscience* **165**, 138-150 (2010) [WB]
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5) Hayashi, K., *et al., J. Neurosci.* **19**, 3918-3925 (1999)
6) Yoshigaki, J. F., *et al., J. Biol. Chem.* **271**, 13130-13134 (1996)
7) Moutot, N. M., *et al., J. Biol. Chem.* **271**, 6567-6570 (1996)
8) Hayashi, K., *et al., J. Neurosci.* **16**, 7161- 7170 (1996)
9) Takei, Y., *et al., J. Cell Biol*. **131**, 1789-1800 (1995)
10) Wakabayashi, K., *et al., Neurosci. Lett.* **138**, 237-240 (1992)
11) Obata, K., *et al., Brain Res.* **375**, 37-48 (1986)

Clone 171B5 is used in these references.

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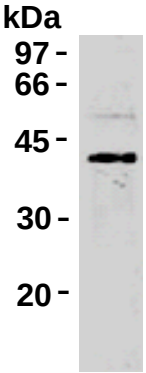


Western blotting analysis of Synaptophysin expression in mouse brain extract using D073-3.

PROTOCOLS:

SDS-PAGE & Western blotting

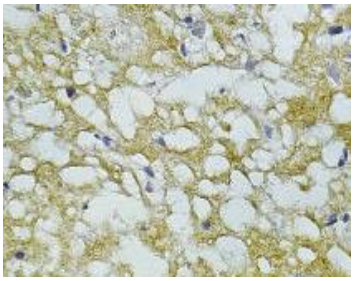
1) Rinse the mouse brain with PBS and suspend with 5 mL of extraction buffer [50 mM HEPES (pH 7.2), 250 mM NaCl, 0.2% NP-40, 5 mM EDTA, 10% glycerol] containing appropriate protease inhibitors.
2) Smash the tissue with homogenizer and sonicate briefly on ice.
3) Centrifuge the tube at 18,000 x g for 15 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold extraction buffer to make 1 mg/mL solution.
4) Mix the sample with equal volume of Laemmli's sample buffer.
5) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
6) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
7) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
8) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
9) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 minutes x 3).
10) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
11) Wash the membrane with PBS-T (10 minutes x 3).
12) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
13) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
14) Expose to an X-ray film in a dark room for 3 minutes.
15) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Mouse brain)

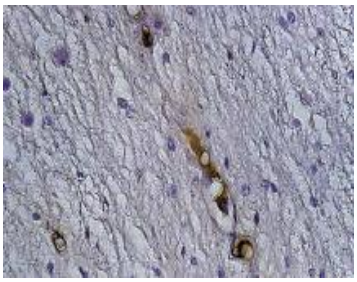
Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Blocking buffer [20 mM HEPES (pH 7.2), 1% BSA, 135 mM NaCl] for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Biotin-conjugated anti-mouse IgG antibody. Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with HRP-conjugated streptavidin. Incubate for 10 minutes at room temperature. Wash as in step 8).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive controls for Immunohistochemistry; Human frontal lobe and spine)



Immunohistochemical detection of Synaptophysin on paraffin embedded section of human frontal lobe.



Immunohistochemical detection of Synaptophysin on paraffin embedded section of human spine.