

Anti-Synaptophysin, clone SY38)

Monoclonal Antibody

Cat. # MAB5258-50UG

Lot # LV1751784

pack size: 50 µg

Store at 2-8°C
DO NOT FREEZE

FOR RESEARCH USE ONLY



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IH	Av, B, F, H, M, R	IgG1k	N/A	M	38 kDa	NM_003179.2

Background

Synaptophysin is a synaptic vesicle glycoprotein with four transmembrane domains weighing 38 kDa. It is present in neuroendocrine cells and in virtually all neurons in the brain and spinal cord that participate in synaptic transmission. The localization and ubiquitous expression of this protein in neuronal tissue makes it an excellent marker for neurons and neuroendocrine tumors. The gene for this protein is located on the X chromosome (Xp11.23-p11.22). The exact function of the protein is unknown: it interacts with the essential synaptic vesicle protein synaptobrevin, but when the synaptophysin gene is experimentally inactivated in animals, they still develop and function normally.

Presentation

Purified mouse monoclonal IgG1k liquid in buffer containing 20 mM sodium phosphate buffer, 5 mg/mL BSA, with 0.01% methylisothiazolone MIT (w/v), pH 7.5.

Concentration

1 mg/mL

Specificity

The antibody reacts with presynaptic vesicles of cerebral and spinal neurons, of neuromuscular endplates and with the retina of man, cow, rat and mouse. The antibody also reacts with vesicles of adrenal medulla and islet cells, and additionally allows specific staining of neuronal, adrenal and neuroepithelial tumors, such as pheochromocytoma, paraganglioma, islet cell tumors (incl. Insulinoma), medullary thyroid carcinoma and diverse pulmonary and gastrointestinal carcinoids. The antibody also stains neurosecretory vesicles of certain culture cells, e.g. of the rat cell line PC-12.

Immunogen

Vesicular fraction of bovine brain

Molecular Weight

38 kDa

Method of Purification

Protein A Purified

Storage and Handling

Stable for 6 months at 2-8°C in undiluted aliquots from date of receipt.

Do Not Freeze.

Control

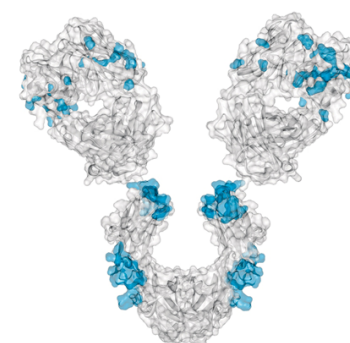
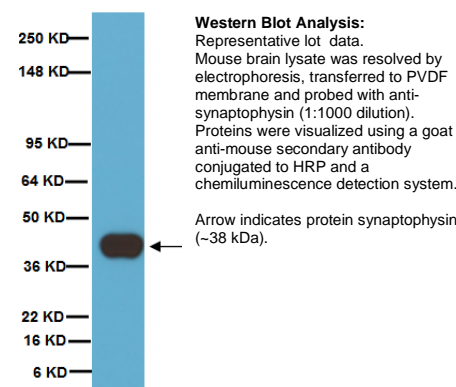
Positive Control: Pancreas

Negative Control: Normal mouse serum

Quality Control Testing

Evaluated by Western Blot on mouse brain lysates.

Western Blot Analysis: 1:1000 dilution of this antibody detected synaptophysin on 10 µg of mouse brain lysates.



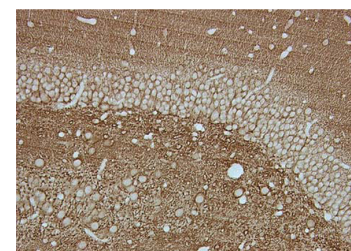
References

1. Tarr, Paul T and Edwards, Peter A. (2008). *J. Lipid Res.* 49: 169-82.
2. Tabuchi, Katsuhiko, et al. (2007). *Science.* 318: 71-6.
3. Masliah, E., et al. (2001). *PNAS.USA.* 98:12245-12250.
4. Wiedenmann, B. & Franke, W. W. (1985). *Cell.* 41, 1017-1028.

Additional Research Applications

Immunohistochemistry: For relatively cytoplasm rich neuroendocrine tumors a final concentration of 1 µg/mL is recommended. A final concentration of 2 µg/mL is recommended for cytoplasm deficient tumors.

Optimal working dilutions must be determined by end user.



APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue)

SPECIES LEGEND: Av Avian B Bovine F Fish H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Rev. A/2009-05-25/MAB5258-50UGCA/JM

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PROTOCOL

Ideal frozen sections (4-5 μ m) are obtained from shock-frozen tissue samples. The frozen sections are air-dried and then fixed with acetone for 5-10 min at -20°C. Excess acetone is allowed to evaporate at room temperature. Material fixed in alcohol and embedded in paraffin can also be used.

It is advantageous to block unspecific binding sites by overlaying the sections with fetal calf serum for 20-30 min at room temperature. Excess of fetal calf serum is removed by decanting before application of the anti-body solution.

Cytocentrifuge preparations of single cells or cell smears are also fixed in acetone. These preparations should, however, not be air-dried, the excess acetone is removed by briefly washing in phosphate-buffered saline (PBS).

Further treatment is then as follows:

1. Overlay the preparation with 10-20 μ L antibody solution and incubate in a humid chamber for 30-60 min at room temperature.
2. Dip the slide briefly in PBS and then wash in PBS 3 times for 3 min each (using a fresh PBS bath in each case).
3. Wipe the margins of the preparation dry, overlay the preparation with 10-20 μ L of a solution of anti-mouse Ig-FITC or anti-mouse IgG-peroxidase solution and allow to incubate in a humid chamber for 30 min at room temperature.
4. Wash the slide in PBS as described above.

The preparation must not be allowed to dry out during any of the steps.

If using an indirect immunofluorescence technique, the preparation should be overlaid with a suitable embedding medium (e.g. Mowiol, Hoechst) and examined under the fluorescence microscope.

If a POD-conjugate has been used as the secondary antibody, the preparation should be overlaid with a substrate solution (see below) and incubated at room temperature until a clearly visible red/brown color develops.

A negative control (e.g. only the secondary antibody) should remain unchanged in color during this incubation period.

Subsequently, the substrate is washed off with PBS and the preparation is stained with hemalum stain for about 1 min. The hemalum solution is washed off with PBS, the preparation is embedded and examined.

Recommendations for paraffin sections:

1. Great care should be taken to remove carefully all traces of paraffin from the sections.
2. Never digest with protease!!
3. We recommend Bouin's fixative for fixing. In the case of formalin-fixed samples, after removing the paraffin, the sections should be incubated longer with the antibody (6-24 h at room temperature; concentration: 0.2-2.0 μ L/mL, depending on the antigen density).
4. With most tissues, the background can be ignored. If background problems occur, a lower concentration of the antibody and longer incubation times are recommended.

Substrate solutions:

Aminoethylcarbazole: Dissolve 2 mg 3-amino-9-ethylcarbazole with 1.2 mL dimethylsulfoxide and add 28.8 mL Tris-HCl, 0.05 mol/l; pH 7.3; and 20 μ L H₂O₂, 3% (w/v). Prepare solution freshly each day.

Diaminobenzidine: Dissolve 25 mg 3,3'-diaminobenzidine with 50 mL Tris-HCl, 0.05 mol/l; pH 7.3; and add 40 μ L H₂O₂, 3% (w/v). Prepare solution freshly each day.

RELATED PRODUCTS (specific)

cat #	description
AB9272	■ Anti-Synaptophysin
MAB368	■ Anti-Synaptophysin
AB9840	■ Anti-Synaptophysin 2
MAB332	■ Anti-Synaptophysin, clone EP10
MAB329	■ Anti-Synaptophysin, clone SP15
MAB5258-20UG	■ Anti-Synaptophysin, clone SY38
IHCR1011-6	■ Anti-Synaptophysin, prediluted, clone SY38
AP124P	■ Goat anti-Mouse IgG, Peroxidase Conjugated, H+L

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 μ m
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 μ m
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 μ m
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

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