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Product datasheet for AM26445AF-N

Synaptophysin (SYP) Mouse Monoclonal Antibody [Clone ID: 171B5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	171B5
Applications:	IHC, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunohistochemistry on parafin sections:** 10 µg/ml. For details see protocol below.
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Purified synaptic vesicle fraction from guinea pig cerebrum
Specificity:	Reacts with human and mouse Synaptophysin.
Formulation:	PBS containing 50% glycerol, pH 7.2. Contains no preservtives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	synaptophysin
Database Link:	Entrez Gene 6855 Human P08247

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Background: Synaptophysin (also referred to as SVP38 and p38) is an acidic Ca2+ binding glycoprotein of about 38 kDa which exist largely in synaptic vesicles. It has 4 transmembrane regions and is reported to be a major cholesterol-binding protein in synaptic vesicles. Synaptophysin may regulate synaptic exocytosis by competing with proteins such as SNAP25 and syntaxins to bind to synaptobrevin (vesicle-associated membrane protein, or VAMP). It may also participate in synaptic endocytosis, which contributes to rapid recycling of synaptic vesicle.

Synonyms: Neuroendocrine Marker

Note: This product was originally produced by MBL International.

Protocol:

SDS-PAGE & Western Blotting

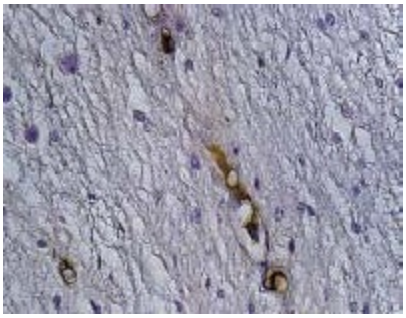
1) Rinse the mouse brain with PBS and suspend with 5 mL of extraction buffer (50 mM Hepes, pH 7.2, 250 mM NaCl, 0.2% NP-40, 5 mM EDTA, 10% glycerol) containing appropriate protease inhibitors.
2) Smash the tissue with homogenizer and sonicate briefly on ice.
3) Centrifuge the tube at 18,000 x g for 15 minutes at 4oC and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 1 mg/mL solution.
4) Mix the sample with an 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/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). 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 4oC.
8) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody to be used will depend on conditions.)
9) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
10) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG 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 times).
12) Wipe excess buffer from the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
13) Remove extra reagent from the membrane by dabbing with a 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 conditions for exposure and development may vary.
(Positive control for Western blotting; mouse brain)

Immunohistochemical staining for paraffin-embedded sections: SAB method

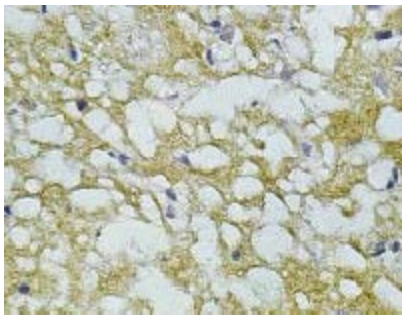
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.

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 Protein Blocking Agent 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 suggest 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 Polyvalent Biotinylated Antibody. Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. 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) Sample is now ready for mounting.
(Positive controls for Immunohistochemistry; Human Frontal lobe, Spine)

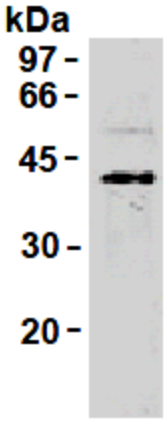
Product images:



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



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



Western blot analysis of Synaptophysin expression in Mouse brain using AM26445AF-N.