

Anti-Bovine Synapsin I, Rabbit IgG Fraction

Introduction

Synapsin I is an actin-binding protein that is localized exclusively to synaptic vesicles and thus serves as an excellent marker for synapses in brain and other neuronal tissues.^{1,2} Synapsin I inhibits neurotransmitter release, an effect that is abolished upon its phosphorylation by Ca^{2+} /calmodulin-dependent protein kinase II.³ Antibodies directed against synapsin I have proven valuable in molecular and neurobiology research, for example, to estimate synaptic density and to follow synaptogenesis.⁴⁻⁶ Molecular Probes offers an affinity-purified anti-bovine synapsin I rabbit polyclonal antibody (A6442). This antibody was isolated from rabbits immunized against bovine brain synapsin I, but is also active against human, rat and mouse forms of the antigen; it has little or no activity against synapsin II. The affinity-purified antibody was fractionated from the serum using column chromatography in which bovine synapsin I was covalently bound to the column matrix. The antibody is suitable for immunohistochemistry, Western blots, enzyme-linked immunoadsorbent assays (ELISAs) and immunoprecipitations.

Materials

Anti-bovine synapsin I is supplied as a lyophilized powder in a unit size of 10 µg. When stored at -20°C or below, the lyophilized antibody should retain full activity for over one year. Reconstitute by adding 50 µL phosphate-buffered saline, pH 7.4 (PBS) to yield a 0.2 mg/mL solution. If the antibody is to be stored at 0–4°C, add sodium azide to a final concentration of 2 mM. For longer storage, divide the solution into aliquots and freeze at -20°C. AVOID FREEZING AND THAWING.

Applications

The following protocols for immunocytochemistry and Western blots offer practical guidelines for the use of anti-synapsin I antibody. Additional applications and details can be found in several of the references provided below.⁷⁻⁹

Immunocytochemistry Rat brain sections, 30 µm thick and fixed with 4% formaldehyde in PBS, are washed several times with PBS and blocked in a solution of 1% horse serum and 0.3% Triton® X-100 for 1 hour. The tissue is then incubated overnight at 4°C in a 1:500 to 1:5000 dilution of the 0.2 mg/mL reconstituted anti-synapsin I antibody. Higher dilutions can be used with tissues with high synaptic density (e.g., cortex and hippocampus); lower dilutions should be used with tissues that have low synaptic density (e.g., peripheral nervous system and spinal cord tissues). Sections are then washed with PBS and incubated for 2 hours in pre-adsorbed fluorophore- or enzyme-labeled anti-rabbit IgG antibody.

Western Blot Analysis Rat cortex homogenate (10–20 µg per lane) is electrophoresed through an SDS-polyacrylamide gel and then transferred to a nitrocellulose or nylon membrane in 20 mM Tris-HCl, 150 mM glycine, pH 8.3, 20% methanol. After transfer is complete, the membrane is washed three times, for 20 minutes each, with 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.05% NaN₃, 0.5% Tween®-20 detergent (incubation buffer). The membrane is then incubated for 2 hours, with shaking, in enough antibody solution to cover it completely. The antibody solution is prepared by diluting the reconstituted anti-synapsin I antibody 1:500 or 1:1,000 in incubation buffer. Once the membrane has incubated in the antibody solution, it is rinsed three times, for 20 minutes each, with incubation buffer and then incubated for 2 hours in ¹²⁵I-protein A diluted in incubation buffer at an activity of 0.1 µCi/mL. The membrane is again rinsed three times, for 20 minutes each, with incubation buffer, dried and analyzed by autoradiography or by cutting out the bands and determining the ¹²⁵I-protein A labeling with a scintillation counter. Alternatively, the anti-synapsin I antibody can be detected using an alkaline phosphatase-conjugated secondary antibody in conjunction with the chromogenic substrates NBT/BCIP.

References

1. Science 226, 1209 (1984); 2. J Cell Biol 96, 1337 (1983); 3. Greengard, P., Benfenati, F. and Valtorta, F. in Molecular and Cellular Mechanisms of Neurotransmitter Release, L. Stjärne, et al., Eds. Raven Press (1994) pp. 31–45; 4. J Neurosci 9, 2151 (1989); 5. J Neurosci 12, 1736 (1992); 6. J Neurosci 11, 1617 (1991); 7. Biol Psychiatry 34, 529 (1993); 8. J Neurosci 14, 301 (1994); 9. Proc Natl Acad Sci USA 88, 2361 (1991).

Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat #	Product Name	Unit Size
A6442	anti-bovine synapsin I, rabbit IgG fraction *affinity purified* *specificity: human, bovine, rat, mouse*.....	10 µg

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