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Antibodies Directed against NMDA Receptors

A-6473 anti–rat NMDA receptor, subunit 2A, rabbit IgG fraction *affinity purified*
A-6474 anti–rat NMDA receptor, subunit 2B, rabbit IgG fraction *affinity purified*
A-6475 anti–rat NMDA receptor, subunit 2C, rabbit IgG fraction *affinity purified*

Quick Facts

Storage upon receipt:
- –20°C
- Avoid freeze-thaw cycles

Introduction

N-methyl-D-aspartate (NMDA) receptors constitute cation channels of the central nervous system that are gated by the excitatory neurotransmitter L-glutamate.[1,2] Activation of NMDA receptors is essential for inducing long-term potentiation (LTP), a form of activity-dependent synaptic plasticity that is implicated in the learning process in animal behavioral models.[3] The biophysical properties of NMDA receptor channels contributing to LTP include Ca^{2+} permeability, voltage-dependent Mg^{2+} block and slow-gating kinetics.[4-7] NMDA receptor channel activities play a role in neuronal development and in disorders such as epilepsy and ischemic neuronal cell death. As targets for ethanol, NMDA receptors may also function in the pathology of alcoholism.[8,9]

In vitro reconstitution experiments with cloned NMDA receptor type 1 subunit and any one of four type 2 subunits, 2A, 2B, 2C and 2D, revealed that the physiological and pharmacological properties of the heteromeric NMDA receptor appear to be imparted by the particular type 2 subunit.[10-13] Subunits 2A and 2B are detected predominantly in the hippocampus and cortex, whereas 2C is found mainly in the cerebellum. Thus, cellular expression profiles of the NMDA receptor subunits may contribute to the biophysical properties of NMDA receptors in specific central neurons.

For neurobiologists, Molecular Probes provides affinity-purified rabbit polyclonal antibodies to NMDA receptor subunits 2A, 2B and 2C. The anti–NMDA receptor 2A antibody (anti-NR2A) was raised against a fusion protein containing amino acid residues 1253–1391 of the C-terminal region of rat brain subunit 2A; this antibody is specific for the ~180,000-dalton 2A subunit from rat, mouse or human. The anti-NR2B antibody was raised against a fusion protein containing amino acid residues 984–1104 of the C-terminal region of rat brain subunit 2B; this antibody is specific for the ~180,000-dalton 2B subunit from rat, mouse or human. The anti-NR2C antibody was raised against a fusion protein containing amino acid residues 25–130 of the N-terminal region of rat brain 2C subunit; this antibody recognizes the 140,000-dalton 2C subunit, as well as the 180,000-dalton 2A and 2B subunits from rat, mouse or human. In Western analysis, this cross-reacting activity can be tolerated because of the easily distinguished molecular weights. Anti-NR2C antibody can also be used for immunoprecipitation from extracted tissues, such as cerebellum, where the 2C subunit is abundant.

The antibodies have been fractionated from sera by column chromatography in which rat NMDA receptor fusion proteins were bound to a matrix. The affinity-purified anti–NMDA receptor antibodies to subunits 2A (A-6473), 2B (A-6474) and 2C (A-6475) are suitable for immunohistochemistry, Western blot analysis, enzyme-linked immunosorbent assay (ELISA) and immunoprecipitation.

Contents and Storage

Each antibody is supplied lyophilized in a unit size of 10 µg. When stored at -20°C or below, the lyophilized antibody should retain full activity for at least a year. Reconstitute by adding 50 µL phosphate-buffered saline (PBS), pH 7.4, 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 into aliquots and freeze at -20°C. AVOID REPEATED FREEZE/THAW CYCLES.

Applications

The following protocols for immunocytochemistry and Western blot analysis offer practical guidelines for the use of anti–NMDA receptor antibodies. Anti-NR2A antibody is highly specific for subunit 2A and does not cross-react with 2B or 2C. Anti-NR2B antibody is highly specific for subunit 2B and does not cross-react with 2A or 2C. In Western blot analysis and immunoprecipitation, anti-NR2C antibody recognizes 2C and cross-reacts with 2A and 2B. Additional applications and details may be found in the references provided below.[14,15]

Immunohistochemistry

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 affinity-purified anti-NMDA receptor antibody. Higher dilutions can be used with tissues that have 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 preadsorbed fluorophore- or enzyme-labeled anti-rabbit antibody (see *Product List*).

For immunoprecipitation of subunits 2A or 2B, 3 µL of the appropriate 0.2 mg/mL reconstituted antibody is sufficient to precipitate all of either subunit from 200 µg of rat brain. For immunoprecipitation of subunit 2C, 3 µL of the 0.2 mg/mL reconstituted anti-NR2C antibody is sufficient to precipitate all of 2C from 200 µg of rat cerebellum.

Western Blot Analysis

Rat cortex homogenate (10–20 µg per lane) is resolved electrophoretically 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% sodium azide, 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 a 0.2 mg/mL solution of the affinity-purified anti–NMDA receptor antibody 1:200 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 ^{125}I–protein A diluted in incubation buf-fer 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 ^{125}I–protein A labeling with a scintillation counter.

References

1. Neuron 12, 529 (1994); **2.** Nature 354, 31 (1991); **3.** J Neurosci 9, 3040 (1989); **4.** Nature 346, 565 (1990); **5.** Nature 325, 529 (1987); **6.** Nature 321, 519 (1986); **7.** Nature 307, 462 (1984); **8.** Mol Pharmacol 45, 324 (1994); **9.** Neurosci Lett 152, 13 (1993); **10.** Mol Pharmacol 45, 540 (1994); **11.** J Biol Chem 268, 2836 (1993); **12.** Mol Pharmacol 44, 851 (1993); **13.** Science 256, 1217 (1992); **14.** Cell 84, 745 (1996); **15.** Brain Res Mol Brain Res 40, 71 (1996).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
A-6473	anti-rat NMDA receptor, subunit 2A, rabbit IgG fraction *affinity purified* *specificity: human, rat, mouse*	10 µg
A-6474	anti-rat NMDA receptor, subunit 2B, rabbit IgG fraction *affinity purified* *specificity: human, rat, mouse*	10 µg
A-6475	anti-rat NMDA receptor, subunit 2C, rabbit IgG fraction *affinity purified* *specificity: human, rat, mouse*	10 µg

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