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Product Information

Anti-phospho-N-Methyl D-Aspartate NR2B Subunit (pTyr^{1472})
produced in rabbit, affinity isolated antibody

Catalog Number **M2442**

Synonym: Anti-phospho-NMDA NR2B (pTyr^{1472})

Product Description
Anti-phospho-N-Methyl D-Aspartate NR2B Subunit (pTyr^{1472}) is produced in rabbit using as immunogen a synthetic phosphorylated peptide derived from the region of NMDA receptor NR2B that is phosphorylated on tyrosine 1472. The antiserum is affinity purified using epitope-specific affinity chromatography with the phosphopeptide column followed by the non-phosphorylated peptide column.

Anti-phospho- N-Methyl D-Aspartate NR2B Subunit (pTyr^{1472}) specifically recognizes rat receptor NR2B subunit (~180 kDa) phosphorylated on tyrosine 1472. It does not crossreact with non-phosphorylated NMDA NR2B. It is likely to detect human, mouse, chicken and *Xenopus* NMDA NR2B based on sequence homology. It is used in immunoblotting, immunocytochemistry, immunoprecipitation and ELISA applications.

The ionotropic glutamate receptor is a prominent ligand-gated and voltage-gated ion channel in excitatory synaptic transmission in the mammalian central nervous system. The glutamate receptor was named the NMDA receptor for its agonist, N-methyl-D-aspartate. Functional NMDA receptors contain an active invariant subunit (NMDAR1) associated with one of several variable regulatory subunits. NR2B is one of the variant regulatory subunits associated with the active subunit NMDAR1 and localized primarily to the hippocampus and forebrain. Changes in the relative expression levels of NMDA NR2A and NMDA NR2B could regulate peak amplitude of NMDA receptor-mediated excitatory postsynaptic potentials and thus modulate the efficiency of synaptic plasticity.[1]

The NMDA channel is also regulated by its phosphorylation. Tyrosine phosphorylation sites on NR2A and NR2B have been identified. Phosphorylation of tyrosine 1472 on the NR2B subunit is largely dependent on Fyn, a member of the tyrosine kinase oncogene family which regulates long-term potentiation, spatial learning, and hippocampal development, and is elevated upon the long-term potentiation (LTP) induction of hippocampal CA1 neurons. The data suggest that tyrosine 1472 phosphorylation of NR2B is important for synaptic plasticity. A phosphorylation of the other tyrosine residues of NR2A and NR2B is also involved in brain development and function.[2]

It is a well-known fact that while phosphorylation of the NMDAR potentiates channel currents, ethanol inhibits the NMDAR in several brain regions. The studies of the mechanism of ethanol inhibition have shown that phosphorylation of both NR2A and NR2B subunits is significantly reduced following *in situ* exposure of hippocampal slices to 100 mM ethanol. Specifically, phosphorylation of tyrosine 1472 on NR2B is reduced at least 20%.[3] These data suggest that ethanol may inhibit the NMDAR via activation of a tyrosine phosphatase. Electrophysiological studies demonstrate that ethanol inhibits NMDAR extracellular field excitatory postsynaptic potentials (fEPSP) slope and amplitude. Inclusion of bpV(phen), a potent phosphotyrosine phosphatase inhibitor, in the recording chamber prior to and during ethanol exposure significantly reduced the inhibitory effect of ethanol on NMDAR fEPSPs. Taken together, these data suggest that phosphatase-mediated dephosphorylation of NMDAR subunits may play an important role in mediating the inhibitory effects of ethanol on the NMDA receptor.[3]

Tyrosine phosphorylation studies in a rat model of inflammation show a correlation of NMDA receptor tyrosine phosphorylation *in vivo* with the development and maintenance of inflammatory hyperalgesia and suggest that signal transduction upstream to NR2B tyrosine phosphorylation involves G-protein-coupled receptors and PKC and Src family protein tyrosine kinases.[4]

Reagent
Supplied as a solution in 10 mM HEPES, pH 7.5, containing 150 mM NaCl, 100 μg/ml BSA and 50% glycerol. Due to the high viscosity of glycerol, the sample must be mixed thoroughly before aliquoting.

Storage/Stability
Store at −20 °C. For extended storage prepare working aliquots. Do not store in frost-free freezers to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile
Immunoblotting: a recommended working dilution of 1:1,000 is determined using rat brain hippocampal tissue homogenate. A band of ~180 kDa is detected. Two additional lower molecular weight immunoreactive bands, ~110 and 65 kDa, are present in the immunoblots of rat brain.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Chen, A., et al., Subtype-dependence of NMDA receptor channel open probability. *J. Neurosci.*, **19**, 6844-6854 (1999).
2. Nakazawa, T., et al., Regulation of NMDA receptor function by Fyn-mediated tyrosine phosphorylation. (English), *Nihon Shinkei Seishin Yakurigaku Zasshi.*, **5**, 165-172 (2002).
3. Alvestad, R.M., et al., Tyrosine dephosphorylation and ethanol inhibition of NMDA receptor function. *J. Biol. Chem.*, **278**, 11020-5 (2003).
4. Guo, W., et al., Tyrosine phosphorylation of the NR2B subunit of the NMDA receptor in the spinal cord during the development and maintenance of inflammatory hyperalgesia. *J. Neurosci.*, **22**, 6208-6217 (2002).

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