

Product Information

MONOCLONAL ANTI-NICOTINIC ACETYLCHOLINE RECEPTOR, $\alpha 1, \alpha 3, \alpha 5$ SUBUNITS, CLONE mAb 35

Purified Rat Immunoglobulin

Product Number **M-217**

Product Description

Monoclonal Anti-Nicotinic Acetylcholine Receptor, $\alpha 1, \alpha 3, \alpha 5$ subunits (rat IgG1 isotype) is derived from the mAb 35 hybridoma produced by the fusion of mouse myeloma cell line S194.1 and splenocytes from an immunized rat using purified nicotinic acetylcholine receptor from the electric eel *Electrophorus* as immunogen.¹ The epitope is located on the extracellular surface of the $\alpha 1$ subunit of the muscle nicotinic acetylcholine receptor.

Monoclonal Anti-Nicotinic Acetylcholine Receptor, $\alpha 1, \alpha 3, \alpha 5$ subunits may be used to localize and detect the $\alpha 1, \alpha 3$, and $\alpha 5$ subunits of the nicotinic acetylcholine receptor (AChR). The antibody recognizes the main immunogenic region (MIR) of the nicotinic acetylcholine receptor. It binds to MIR on the extracellular surface of the $\alpha 1$ subunit of muscle acetylcholine receptors and to homologous regions on $\alpha 3$ and $\alpha 5$ subunits of neuronal AChRs. The antibody can be used in chick, eel, and human tissue, but not on *Xenopus* tissue. It detects $\alpha 1$ in all species tested except *Xenopus*, detects $\alpha 5$ in chicken and human, and $\alpha 3$ in human. The antibody binds to denatured chick $\alpha 5$ and strongly with native $\alpha 1$.^{2, 3} It does not bind well to denatured chick $\alpha 3, \alpha 4, \alpha 7, \alpha 8$, and $\beta 2$.⁴ The antibody may also bind $\beta 3$ due to the homology of the main immunogenic region. The antibody may be used for RIA, immunohistochemistry,⁵ and immunoblotting.⁶

Nicotinic acetylcholine receptors are members of a gene superfamily of ligand-gated ion channels which includes the homologous GABA_A receptors, glycine receptors and 5-HT₃ serotonin receptors,^{7, 8} but not the structurally dissimilar ligand-gated ion channels comprising the glutamate⁹ or ATP receptors.^{10, 11} It is likely that all receptors in the AChR superfamily are comprised of five homologous subunits oriented around a central ion channel.¹²

AChRs were first characterized in the skeletal muscles and their structural properties were initially character-

ized in using AChRs from the homologous electric organ tissue of the *Torpedo* rays,^{12, 13, 14} The functional and structural characterization of neuronal AChRs developed later due to their lower concentrations in more heterogenous tissues.

Most, if not all, subunits that form the AChRs have now been cloned and expressed. Although more is known now about the structure and function of the neuronal AChRs still little is known about the physiological roles of the many subtypes.

AChRs are now being associated with a growing number of diseases. Thus more research is required to determine the physiological function and role of the AChR subtypes as well as the receptors themselves in the hopes of discovering new treatments for these pathologies.

Reagents

Monoclonal Anti-Nicotinic Acetylcholine Receptor, $\alpha 1, \alpha 3, \alpha 5$ subunits is supplied at a concentration of approximately 5 mg/mL in 20 mM sodium phosphate, pH 7.2, containing 150 mM sodium chloride and 0.05% sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

The recommended working dilution is 1:3,000 – 1:30,000 for immunoblotting and immunohistochemistry.

The moles of *Torpedo californica* electric organ muscle-type receptor α -bungarotoxin binding sites bound per liter of antibody stock solution is measured using a liquid phase RIA as described by Lindstrom, J., et al.,¹⁵ and by a solid phase RIA using goat anti-rat IgG bound to microwell plates. Antibody dilutions for these assays were 1:5,000 – 1:1,000,000. Lot specific titers are given in the certificate of analysis.

Note: In order to obtain the best results in various techniques and preparations we recommend determining optimal working dilution by titration.

References

1. Tzartos, S. et al., J. Biol. Chem., **256**, 8635-8645 (1981).
2. Das, M., and Lindstrom, J., Biochem. Biophys. Res. Commun., **165**, 865-871 (1989).
3. Saedi, M.S., FEBS. Lett., **267**, 55 (1990).
4. Conroy, W., et al., Neuron, **9**, 1-20 (1992).
5. Keyser, K., et al., Vis. Neurosci., **1**, 349 (1988).
6. Wang, F., et al., J. Biol. Chem., **271**, 17656 (1996).
7. Betz, H., Neuron, **5**, 383 (1990).
8. Barnard, E., Trends Biol. Sci., **17**, 368 (1992).
9. Seeburg, P., Trends Neurosci., **16**, 359 (1993).
10. Brake, A., et al., Nature, **371**, 519 (1994).
11. Valera, S., et al., Nature, **371**, 516 (1994).
12. Lindstrom, J., "Ion Channels", Vol. 4, Narahashi, T. (ed), Plenum Press, New York (1996).
13. Changeux, J., Fidia Research Foundation, Neuroscience Award Lectures, **4**, 21 (1990).
14. Karlin, A. and Akabas, M., Neuron, **15**, 1231 (1995).
15. Lindstrom, J., et al., Production and Assay of Antibodies to Acetylcholine Receptors, in Meth. Enzymol., vol. 74, Langone, J.J., and Van Vunakis, H., (eds.) Academic Press, pp 432-460 (1981).

Sold with the permission of the Salk Institute.

kaa/sms 07/02

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.