upstate | CHEMICON | Linco
THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

GUINEA PIG ANTI-NICOTINIC ACETYLCHOLINE RECEPTOR α4 POLYCLONAL ANTIBODY

CATALOG NUMBER: AB5590 **QUANTITY:** 50 µL

LOT NUMBER:

SPECIFICITY: Nicotinic Acetylcholine Receptor α4 (nAChR α4). The antibody stains nerve fibers and cell bodies in untreated animals. The staining pattern for the immunoreactive cell bodies obtained with AB5590 corresponds to the pattern described using *in situ* hybridization with probes to rat nAChR receptor mRNA. (Wada et al., 1989, 1990; Duvoisin et al., 1989; Dineley-Miller and Patrick, 1992; Seguela et al., 1993) as well as using antisera to nAChR (Mason, 1985; Swanson et al., 1987; Schroder et al., 1989; Bravo and Karten, 1992; Okuda et al., 1993; Dominguez del Toro et al., 1994; Nakayama et al., 1995; Goldner et al., 1997; Rogers et al., 1998; Sorenson et al., 1998; Arroyo-Jiménez et al., 1999). Strong nAChR α4 immunoreactivity is demonstrated in the Purkinje cells of the cerebellum, in neurons of the cerebral cortex, striatum, brainstem and spinal cord.

IMMUNOGEN: Synthetic peptide corresponding to amino acids 568-588 of the rat nAChR α4 protein.

APPLICATIONS: Immunohistochemistry: 1:1,000-1:2,000 using a Cy3 conjugated secondary antibody. The tissue was fixed in a paraformaldehyde/picric acid-mixture (4% paraformaldehyde and 0.4% picric acid in 0.16 M sodium phosphate buffer, pH 6.9). See application notes on back page.
Western blot: not tested
Optimal working dilutions must be determined by end user.

SPECIES REACTIVITIES: Rat. It is expected that the antibody will also react with mouse and human based on sequence homology. Other species have not yet been tested.

FORMAT: Guinea pig serum.

PRESENTATION: Liquid. Contains no preservative.

STORAGE/HANDLING: Maintain frozen at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

RELATED REFERENCES:

Arroyo-Jiménez MM, Bourgeois JP, Marubio LM, Le Sourd AM, Ottersen OP, Rinvik E, Fairen A, and Changeux JP (1999) Ultrastructural localization of the alpha4-subunit of the neuronal acetylcholine nicotinic receptor in the rat substantia nigra. *J. Neurosci.* **19**:6475-6487.

Bravo H, Karten HJ (1992) Pyramidal neurons of the rat cerebral cortex, immunoreactive to nicotinic acetylcholine receptors, project mainly to subcortical targets. *J. Comp. Neurol.* **320**:62-68.

Deneris ES, Boulter J, Swanson LW, Patrick J, Heinemann S (1989) β_3: a new member of nicotinic acetylcholine receptor gene family is expressed in brain. *J. Biol. Chem.* 264:6268-6272.

Dineley-Miller K, Patrick J (1992) Gene transcripts for the nicotinic acetylcholine receptor

subunit, α_4, are distributed in multiple areas of the rat central nervous system. *Mol. Brain Res.* **16**:339-344.

Dominguez del Toro E, Juiz JM, Peng X, Lindstrom J, Criado M (1994) Immunocytochemical localization of the 7 subunit of the nicotinic acetylcholine receptor in the rat central nervous system. *J. Comp. Neurol.* **349**:325-342.

Duvoisin RM, Deneris ES, Patrick J, Heinemann S (1989) The functional diversity of the neuronal nicotinic acetylcholine receptors is increased by a novel subunit: α_4. *Neuron* **3**:487-496.

Goldman D, Deneris E, Luyten W, Kochhar A, Patrick J, Heinemann S (1987) Members of a nicotinic acetylcholine receptor gene family are expressed in different regions of the mammalian central nervous system. *Cell* **48**:965-973.

Goldman D, Deneris E, Luyten W, Kochhar A, Patrick J, Heinemann S Elliott KJ, Ellis SB, Berckhan KJ, Urrutia A, Chavez-Noriega LE, Johnson EC, Velicelebi G, and Harpold MM (1996) Comparative structure of human neuronal alpha 2-alpha 7 and beta 2-beta 4 nicotinic acetylcholine receptor subunits and functional expression of the alpha 2, alpha 3, alpha 4, alpha 7, beta 2, and beta 4 subunits. *J. Mol. Neurosci.* **7**:217-228.

Goldner FM, Dineley KT, Patrick JW (1997) Immunohistochemical localization of the nicotinic acetylcholine receptor subunit α_6 to dopaminergic neurons in the substantia nigra and ventral tegmental area. *NeuroReport* **8**:2739-2742.

Hill JA, Zoli M, Bourgeois JP, Changeux JP (1993) Immunocytochemical localization of a neuronal nicotinic receptor: the β_2 subunit. *J. Neurosci.* **13**:1551-1568.

Mason WT (1985) Staining of magnocellular nuclei of the rat hypothalamus by a monoclonal antibody directed against the α-subunit of the nicotinic cholinergic receptor. *Neurosci. Lett.* **59**:89-95.

Monteggia LM, Gopalakrishnan M, Touma E, Idler KB, Nash N, Arneric SP, Sullivan JP, and Giordano T (1995) Cloning and transient expression of genes encoding the human alpha 4 and beta 2 neuronal nicotinic acetylcholine receptor (nAChR) subunits. *Gene* **155**:189-193.

Nakayama H, Shioda S, Okuda H, Nakashima T, Nakai Y (1995) Immunocytochemical localization of nicotinic acetylcholine receptor in rat cerebral cortex. *Mol. Brain Res.* **32**:321-328.

Okuda H, Shioda S, Nakay Y, Nakayama H, Okamoto M, Nakashima T (1993) Immunocytochemical localization of nicotinic acetylcholine receptor in rat hypothalamus. *Brain Res.* **625**:145-151.

Rogers SW, Gahring LC, Collins AC, Marks M (1998) Age-related changes in neuronal nicotinic acetylcholine receptor subunit α_4 expression are modified by long-term nicotine administration. *J. Neurosci.* **18**:4825-4832.
Schroder H, Zilles K, Maelicke A, Hajos F (1989) Immunohisto- and cytochemical localization of cortical nicotinic cholinoceptors in rat and man. *Brain Res.* **502**:287-295.

Seguela P, Wadiche J, Dineley-Miller K, Dani JA, Patrick JW (1993) Molecular cloning, functional properties, and distribution of rat brain-7. A nicotinic cation channel highly permeable to calcium. *J. Neurosci.* **13**:596-604.

Sorenson EM, Shiroyama T, Kitai ST (1998) Postsynaptic nicotinic receptors on dopaminergic neurons in the substantia nigra pars compacta of therat. *Neuroscience* **87**:659-673.

Steinlein O, Weiland S, Stoodt J, and Propping P (1996) Exon-intron structure of the human neuronal nicotinic acetylcholine receptor alpha 4 subunit (CHRNA4). *Genomics* **32**:289-294.

Swanson LW, Simmons DM, Whiting PJ, Lindstrom J (1987) Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. *J. Neurosci.* **7**:3334-3342.

Wada E, Wada K, Boulter J, Deneris E, Heinemann S, Patrick J, Swanson LW (1989) Distribution of alpha 2, alpha 3, alpha 4, and beta 2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *J. Comp. Neurol.* **284**:314-335.

Wada E, McKinnon D, Heinemann S, Patrick J, Swanson LW (1990) The distribution of mRNA encoded by a new member of the neuronal nicotinic acetylcholine receptor gene family (alpha 5) in the rat central nervous system. *Brain Res.* **526**:45-53.

Zoli M, Lena C, Picciotto MR, Changeux JP (1998) Identification of four classes of brain nicotinic receptors using beta2 mutant mice. *J. Neurosci.* **18**:4461-4472.

APPLICATION NOTES FOR AB5590

IMMUNOHISTOCHEMISTRY

Male Sprague-Dawley rats (b.wt. 100-150g) were anesthetized with sodium pentobarbital and perfused via the ascending aorta with 50 mL of Ca2+-free Tyrode+s solution followed by a formalin-picric acid fixative (4% paraformaldehyde with 0.4% picric acid in 0.16 M phosphate buffer, pH 6.9) for 6 minutes. Tissues were rapidly dissected out, postfixed in the same fixative for 90 minutes and rinsed for at least 24 hours in 0.1 M phosphate buffer (pH 7.4) containing 10% sucrose. Sections were cut (14 um) in a cryostat and incubated at 4°C overnight with AB5590 (1:1,000-1:2,000). After rinsing in PBS sections were incubated for 60 minutes at room temperature with Cy3-conjugated secondary antibodies. After mounting in a mixture of PBS and glycerol (1:3) containing 0.1% p-phenylenediamine, sections were examined with a Nikon Microphot-SA epifluorescence microscope.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

upstate | CHEMICON | Linco

THE EXPERTISE OF UPSTATE®, CHEMICON® AND LINCO® IS NOW A PART OF MILLIPORE

28820 Single Oak Drive • Temecula, CA 92590
Technical Support: T: 1-800-MILLIPORE (1-800-645-5476) • F: 1-800-437-7502
www.millipore.com