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Product Information

Anti-Nerve Growth Factor-β
produced in goat, affinity isolated antibody

Catalog Number **N8773**

Synonym: Anti-NGF-β

Product Description

Anti-Nerve Growth Factor-β is produced in goat using as immunogen purified recombinant rat Nerve Growth Factor-β, expressed in *Sf*21 insect cells. Affinity isolated antibody is obtained from goat Anti-Nerve Growth Factor-β antiserum by immunospecific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide immunogen.

Anti-Nerve Growth Factor-β recognizes recombinant rat Nerve Growth Factor-β by various immunochemical techniques including immunoblotting, neutralization, capture ELISA, and immunohistochemistry.

Nerve Growth Factor (NGF) was first discovered in mouse sarcomas and described as a diffusable agent which promoted fiber outgrowth of sensory neurons in chick embryos.[1-3] It has also been purified from snake venom[4] and from mouse salivary glands.[5] NGF was originally isolated as a 7S complex composed of three non-convalently linked subunits, α, β, and γ. It is now known that both the α and γ subunits of NGF are members of the kallikrein family of serine proteases while the β subunit, called β-NGF or 2.5S NGF, demonstrates all the biological activities ascribed to NGF. In solution, β-NGF is a non-disulfide linked homodimeric polypeptide. Recombinant rat β-NGF[6] consists of two 120 amino acid residues with a predicted molecular mass of ~13.2 kDa.

Human, mouse, and rat β-NGF share ~90% sequence homology with full cross reactivity in biological actions. Nerve Growth Factor (NGF), a neurotropic agent, is a member of the neurotrophin family of cytokines, which also includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4).[7-8] It is the most characterized member of neurotrophin family and exhibits activity in many different tissues. NGF induces the formation of neurite-like filaments from chick embryo dorsal root ganglia[2] and from rat PC12 pheochromcytoma cells. *In vivo* NGF is involved in fetal development[9] and nerve regeneration.[10] In the peripheral nervous system NGF is critical to the development of sympathetic and certain sensory nerves.[11] In the central nervous system NGF has a tropic role in the development and maintenance of cholinergic neurons of the basal forebrain. It also plays a role in adult CNS tissues in injury repair and neural regeneration.[12] In addition, NGF seems to have a significant role in balancing the interplay among nervous, immune, and endocrine systems. NGF has biological effects on non-neuronal tissues. It plays an important role in the regulation of the immune system. NGF is mitogenic for the factor-dependent human erythroleukemic cell line, TF-1. NGF will enhance histamine release by basophils and mast cells in response to various stimuli. It also induces the growth and differentiation of human B-lymphocytes and suppresses apoptosis in murine peritoneal neutrophils. In response to various stimuli, NGF released from mast cells may elicit a variety of local actions, including neurotransmitter up-regulation, pain and injury perception, and mast cell increase in number and size.

NGF responsive cells include mast cells, lymphocytes, neutrophils, keratinocytes, melanocytes, and cellular elements of specific endocrine glands. It has been shown that NGF is a strong stimulator of breast cancer cell proliferation by the tyrosine kinase activity of TrkA and the MAP-kinase pathway, suggesting that NGF plays a specific role in the initiation and progression of breast cancer.[13]

Cellular receptors for NGF have been found in a variety of cell lines and tissues, including cholinergic neurons of the brain[14] and Schwann cells of damaged nerve axons.[10] Two kinetic types of NGF receptors have been identified from peripheral neurons, neuroblastoma cells,[15] and PC12 cells.[16] NGF receptors are designated as type I (high affinity) and type II (low affinity).[17]

Reagent
Lyophilized from 0.2 μm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline to produce a 0.1 mg/ml stock solution of antibody.

Storage/Stability
Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots. Avoid repeated freezing and thawing. Do not store in frost-free freezer.

Product Profile
Neutralization: Anti-Nerve Growth Factor-β neutralizes the bioactivity of recombinant rat β-NGF, recombinant human β-NGF, and recombinant mouse β-NGF. To measure this biological activity,[18] recombinant rat β-NGF is incubated with various concentrations of the antibody for 1 hour at room temperature in a 96 well plate. Following this preincubation, human erythroleukemic cells, TF-1, are added to the wells. The assay mixture, in a total volume of 100 μl, containing antibody at concentrations from 0.001 to 1 μg/ml, recombinant rat β-NGF (4 μg/ml), and TF-1 cells (1.0×10^5 cells/ml), is incubated at 37 °C in a humidified CO_2 chamber for 48 hours. ^{3}H-thymidine is added during the last 4 hours of the incubation. The cells are harvested onto glass fiber filters and the ^{3}H-thymidine incorporated into the DNA is measured.

The Neutralization $Dose_{50}$ (ND_{50}) is defined as that concentration required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

The Neutralization $Dose_{50}$ (ND_{50}) for Anti-Nerve Growth Factor-β is approximately 0.02-0.1 μg/ml in the presence of 10.0 μg/ml of recombinant rat β-NGF using the human erythroleukemic TF-1 cell line.

The exact concentration of antibody required to neutralize recombinant rat β-NGF activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity.

Capture ELISA:, the antibody can be used as a capture antibody in a rat β-NGF ELISA in combination with biotinylated rat β-NGF affinity purified polyclonal detection antibody. Using plates coated with 100 μl/well of the capture antibody at 0.2-0.8 μg/ml, in combination with 100 μl/well of the detection antibody (100 ng/ml), an ELISA for sample volumes of 100 μl (range of 31.2-2000 pg/ml) can be obtained.

There is less than 1% cross-reactivity with recombinant human β-NGF. No significant cross-reactivity or interference was seen with recombinant human BDNF, recombinant human CNTF, recombinant human NT-3, recombinant human GDNF, recombinant human NT-4, recombinant rat GDNF, recombinant rat GDNF Rα, and recombinant rat CNTF.

Immunoblotting: a working antibody concentration of 0.1-0.2 μg/ml antibody is recommended. The detection limit for recombinant rat β-NGF is ~2 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry: a working antibody concentration of 5-15 μg/ml is recommended to detect β-NGF in cells and tissues.

Note: In order to obtain best results in different techniques and preparations, determination of optimal working dilutions by titration test is recommended.

Endotoxin: <0.1 EU (endotoxin units)/μg antibody determined by the LAL method.

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