Recombinant Rat β-NGF

Catalog Number: 7815-NG

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived rat beta-NGF protein Ser122-Gly241 Accession # P25427
N-terminal Sequence Analysis	Ser122
Predicted Molecular Mass	13.4 kDa

SPECIFICATIONS

SDS-PAGE	11-12 kDa, reducing conditions
Activity	Measured in a cell proliferation assay using TF-1 human erythroleukemic cells. Kitamura, T. *et al.* (1989) J. Cell Physiol. **140**:323. The ED_{50} for this effect is 0.3-1.8 ng/mL.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with BSA as a carrier protein. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in PBS containing at least 0.1% human or bovine serum albumin.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

NGF (Nerve growth factor; also known as β-NGF and 2.5S NGF) is a 13-14 kDa secreted member of the neurotrophin family, cysteine knot superfamily of molecules (1-3). It is a noncovalent dimer expressed by a wide variety of cells including adipocytes (4), eosinophils (5), mast cells (DC) (6), neurons (7), $CD4^+$ and $CD8^+$ T cells (8), activated astrocytes (9), keratinocytes (10), rodent submandibular gland epithelium (11) and activated microglia plus Schwann cells (12). Rat β-NGF is synthesized as a precursor that is 241 amino acids (aa) in length (13, 14). Based on mouse, it contains an 18 aa signal sequence, a 103 aa cleavable proregion, a 118 aa mature region (aa 122-239) and a two aa C-terminal propeptide (13-16). Based on mouse, there is likely to be a 307 aa isoform that utilizes an upstream alternative start site (14, 16, 17). Although proteolytic processing typically occurs intracellularly, proNGF is well recognized to be secreted, where it either undergoes extracellar proteolytic processing, or remains intact to act as a bioactive isoform of NGF (3, 7, 18, 19, 20). Mature rat NGF shares 92% and 96% aa sequence identity with human and mouse NGF, respectively. It should be noted that β-NGF and NGF are synonymous. NGF was originally isolated from the mouse submaxillary/submandibular gland as part of a 7S/140 kDa complex that contained three subunits; an inactive kallikrein molecule (α-subunit), a potentially active different kallikrein molecule (γ-subunit) and a β-subunit (NGF). Other than in rodent, this complex does not exist, and the two kallikreins are not believed to contribute to NGF proteolytic processing. Their presence may simply reflect an incidental release of kallikrein molecules during NGF secretion (3, 20).

NGF has a number of functions, some of which seem at odds with each other. Although it has been reported to have direct neurotrophic activity in the CNS, this is likely due to the effects of proNGF rather than NGF (21). Outside the CNS and during development, NGF activity reportedly ensures that the proper number of heat-sensitive C-fibers form, and that they maintain their sensitivity to heat through TRPV1 expression (22). And in the immune system, NGF promotes mast cell survival at the expense of proliferation (23). During inflammation, NGF is also reportedly up-regulated by Schwann cells. Here, it acts on local neurons, inducing neuronal TNF-α secretion, which feeds-back on TNF type II receptors on neurons and promotes survival (12). By contrast, NGF action on select cell lines has been shown to induce apoptosis, and this has led to some confusion as to the mechanism of NGF action (24). To date, there are two receptors for NGF (at least three for proNGF) (2, 25-27). They are p75NTR and TrkA. It would appear at this time that the p75:TrkA ratio drives the various outcomes associated with NGF exposure (21, 25).

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