Sterile

#5221

Human β-Nerve Growth Factor (hβ-NGF)

Cell Signaling TECHNOLOGY®

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rev. 03/09/20

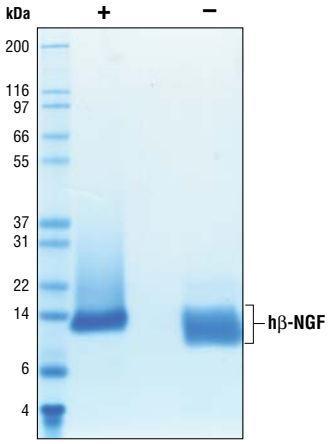
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant human β-NGF (hβ-NGF) Ser122-Ala241 (Accession #NP_002497) was expressed in human 293 cells at Cell Signaling Technology.

Molecular Characterization: Based on amino acid sequencing, greater than 85% of recombinant hβ-NGF starts at Ser122 (SSSHP) and has a calculated MW of 13,494. The remainder starts at Ser124 (SHPIF). DTT-reduced and non-reduced protein migrate as 13 kDa non-disulfide linked homodimers.

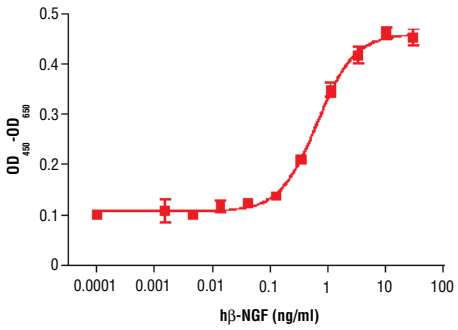
Endotoxin: Less than 0.01 ng endotoxin/1 μg hβ-NGF.

Purity: >98% as determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant hβ-NGF. All lots are greater than 98% pure.

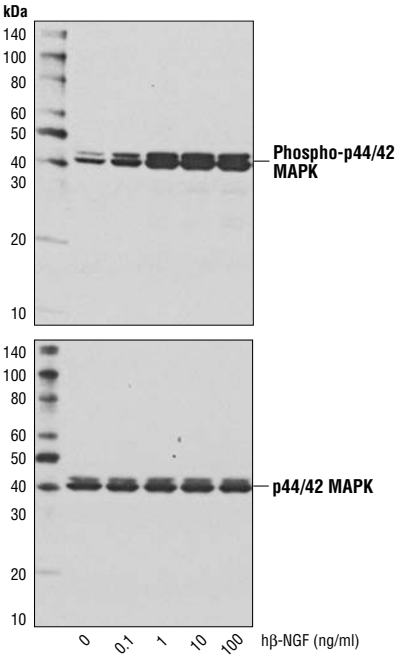


The purity of recombinant hβ-NGF was determined by SDS-PAGE of 6 μg reduced (+) and non-reduced (-) recombinant hβ-NGF and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hβ-NGF was determined in a TF-1 cell proliferation assay. The ED_{50} of each lot is between 0.5-1.5 ng/ml.



◀ *The proliferation of TF-1 cells treated with increasing concentrations of hβ-NGF was assessed. After 48 hour treatment with hβ-NGF, cells were incubated with a tetrazolium salt and the OD_{450}-OD_{650} was determined.*



Western blot analysis of extracts from TF-1 cells untreated or treated with hβ-NGF for 10 minutes, using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP™ Rabbit mAb #4370 (upper) and p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 (lower).

Formulation: With carrier: Lyophilized from a 0.22 μm filtered solution of PBS, pH 7.2 containing 20 μg BSA per 1 μg hβ-NGF.

Carrier free: Lyophilized from a 0.22 μm filtered solution of PBS, pH 7.2.

Reconstitution:
With carrier: Add sterile PBS or PBS containing 1% bovine or human serum albumin or 5-10% FBS to a final hβ-NGF concentration of greater than 50 μg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile PBS or PBS containing protein to minimize absorption of hβ-NGF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hβ-NGF should be greater than 50 μg/ml.

Storage: Stable in lyophilized state at -20°C for 1 year after receipt. Sterile stock solutions reconstituted with carrier protein are stable at 4°C for 2 months and at -20°C for 6 months. Avoid repeated freeze-thaw cycles.

Maintain sterility. Storage at -20°C should be in a manual defrost freezer.

Applications: Optimal concentration for the desired application should be determined by the user.

Background: β-NGF is the prototypical member of the neurotrophin family of growth factors (1). β-NGF is involved in neuronal survival, differentiation and growth (1,2). Outside of the nervous system, NGF is produced by a variety of immune cells, including B cells, T cells, monocytes and mast cells (2,3). β-NGF binds to and signals through two distinct receptors, TrkA and $p75^{NTR}$ (1,2). Cellular responses induced by NGF are modulated by receptor expression. For example, TrKA leads to the inhibition of apoptosis and neuronal differentiation. In contrast, signaling through $p75^{NTR}$ in the absence of TrkA induces cell death. (1,2). NGF signaling via TrkA is characterized by activation of the PI3K/Akt and PLCγ pathways (1,2). NGF signaling via $p75^{NTR}$ induces JNK and NFκB activation (1,2). Aberrant NGF signaling may be linked to the onset of Alzheimer disease (4,5).

Background References:

(1) Bibel, M. and Barde, Y.A. (2000) *Genes Dev* 14, 2919-37.

(2) Huang, E.J. and Reichardt, L.F. (2003) *Annu Rev Biochem* 72, 609-42.

(3) Frossard, N. et al. (2004) *Eur J Pharmacol* 500, 453-65.

(4) Jiang, Y. et al. (2007) *J Immunol* 179, 6297-304.

(5) Matrone, C. et al. (2009) *Proc Natl Acad Sci USA* 106, 11358-63.

(6) Matrone, C. et al. (2008) *Proc Natl Acad Sci USA* 105, 13139-44.