Sterile

#5237

Human Neurotrophin-3 (hNT-3)

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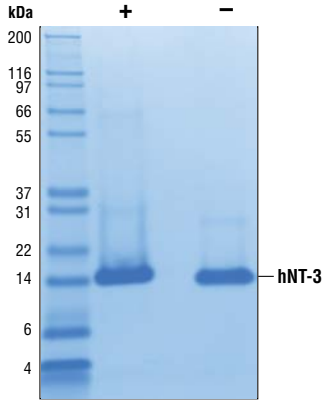
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant human NT-3 (hNT-3) Tyr139-Thr257 (Accession #NP_002518) was produced in *E.coli* at Cell Signaling Technology.

Molecular Characterization: Recombinant hNT-3 does not have a Met on the amino terminus and has a calculated MW of 13,625. DTT-reduced and non-reduced protein migrate as 14 kDa polypeptides. The expected amino-terminal YAEHK of recombinant hNT-3 was verified by amino acid sequencing.

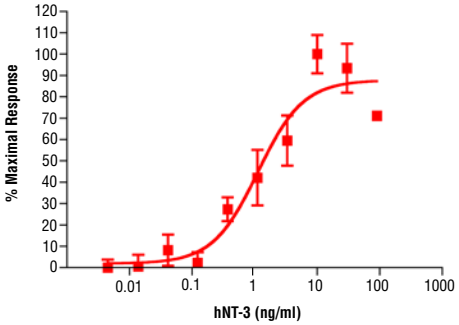
Endotoxin: Less than 0.01 ng endotoxin/1 µg hNT-3.

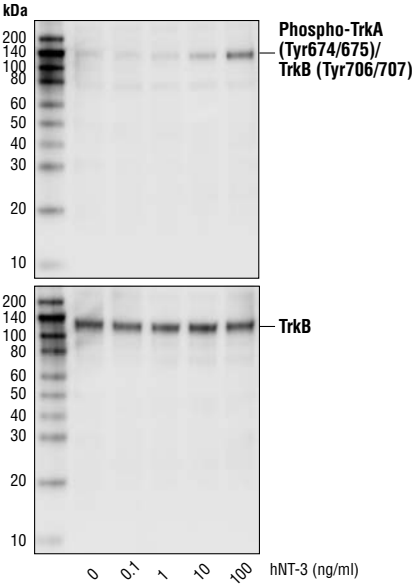
Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hNT-3. All lots are greater than 98% pure.



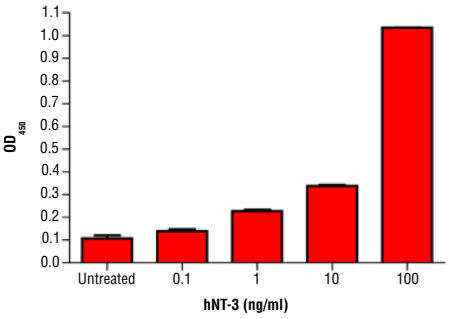
The purity of recombinant hNT-3 was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hNT-3 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hNT-3 was determined in a TrkC-transfected NIH/3T3 cell proliferation assay. The ED_{50} of each lot is between 0.5 - 5 ng/ml.





Western blot analysis of extracts from TrkB-transfected NIH/3T3 cells untreated and treated with hNT-3 for 5 minutes, using Phospho-TrkA (Tyr674/675)/TrkB (Tyr706/707) (C50F3) Rabbit mAb #4621 (upper) or TrkB (80E3) Rabbit mAb #4603 (lower).



The ability of hNT-3 to induce phosphorylation of TrkB was assessed. After starvation, TrkB transfected NIH/3T3 cells were treated with increasing concentrations of hNT-3 for 5 minutes. Cells were lysed, and phospho-TrkB was quatified using PathScan® Phospho-TrkB (panTyr) Sandwhich ELISA Kit #7108. OD_{450}-OD_{650} is shown.

◀ *The proliferation of TrkC-transfected NIH/3T3 cells treated with increasing concentrations of hNT-3 was assessed. After 24 hr treatment, cells were labeled with BrdU for 4 hrs. BrdU incorporation was determined by ELISA and the OD_{450}-OD_{690} was determined.*

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of 20 mM phosphate, pH 8.0 containing 500 mM NaCl and 20 µg BSA per 1 µg hNT-3.

Carrier free: Lyophilized from a 0.22 µm filtered solution of 20 mM phosphate, pH 8.0 containing 500 mM NaCl.

Reconstitution:
With carrier: Add sterile PBS, or PBS containing 1% bovine or human serum albumin or 5-10% FBS to a final hNT-3 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 hNT-3 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hNT-3 should be greater than 50 µg/ml.

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

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

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

Background: NT-3 is a member of the structurally related neurotrophin family of proteins, which includes β-NGF, BDNF and NT-4 (1). NT-3 is expressed in a number of cell types including neuronal cells, eosinophils, and melanocytes (1-3). NT-3 is required for the development of peripheral sensory neurons (4). NT-3 is secreted from cells as a precursor protein, which is proteolytically cleaved into the mature form (1). NT-3 signaling is mediated through two distinct receptors, the neurotrophin receptor p75NTR and the Trk tyrosine kinase receptor TrkC. While all neurotrophins bind to the p75NTR receptor, NT-3 preferentially binds to TrkC and, with lesser affinity, TrkB (1).

Background References:

(1) Huang, E.J. and Reichardt, L.F. (2001) *Annu Rev Neurosci* 24, 677-736.

(2) Noga, O. et al. (2003) *Clin Exp Allergy* 33, 649-54.

(3) Truzzi, F. et al. (2008) *J Invest Dermatol* 128, 2031-40.

(4) Tessarollo, L. et al. (1994) *Proc Natl Acad Sci U S A* 91, 11844-8.