

Human Neuregulin-1 (hNRG-1)

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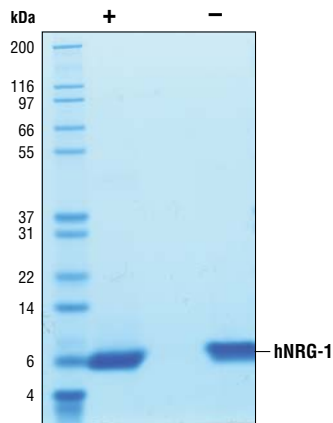
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Source: Recombinant human NRG-1 (hNRG-1) Thr176-Lys238 (Accession #NP_001153480) was produced in *E. coli* cells at Cell Signaling Technology.

Molecular Characterization: Recombinant hNRG-1 does not have a Met on the amino terminus and has a calculated MW of 7,284. DTT-reduced and non-reduced protein migrate as 6 kDa polypeptides. The expected amino-terminal TSHLV of recombinant hNRG-1 was verified by amino acid sequencing.

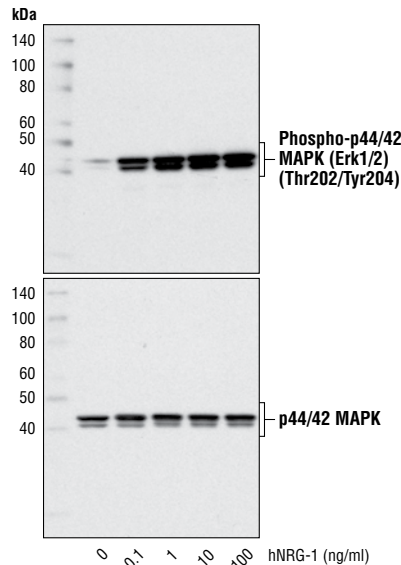
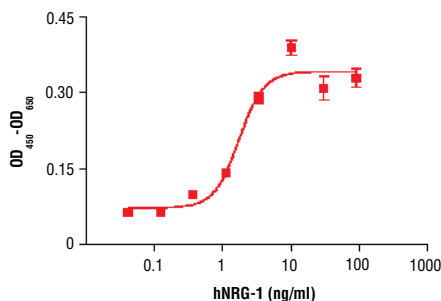
Endotoxin: Less than 0.01 ng endotoxin/1 µg hNRG-1.

Purity: >98% as determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hNRG-1. All lots are greater than 98% pure.



The purity of recombinant hNRG-1 was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant hNRG-1 and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hNRG-1 was determined in a cell proliferation assay using MCF7 cells. The ED₅₀ of each lot is between 1-4 ng/ml.



Western blot analysis of extracts from MCF7 cells, untreated or treated with hNRG-1 for 7 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 20 mM citrate, pH 3.0 containing 100 mM NaCl and 20 µg BSA per 1 µg hNRG-1.

Carrier free: Lyophilized from a 0.22 µm filtered solution of 20 mM citrate, pH 3.0 containing 100 mM NaCl.

Reconstitution:

With carrier: Add sterile 20 mM citrate, pH 3.0 to a final hNRG-1 concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile 20 mM citrate, pH 3.0, or 20 mM citrate, pH 3.0 containing protein to minimize absorption of hNRG-1 to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hNRG-1 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: NRG-1, a member of the EGF family, is involved in heart, mammary and nervous system development (1). NRG-1 is expressed by mammary epithelial, vascular endothelial and neuronal cells (2,3). At least 15 NRG-1 splice variants are known (1). These variants differ in EGF domains (α or β variants), amino-terminal splicing sites, and incorporation of exons encoding integral membrane regions (1). NRG-1 can induce or inhibit the proliferation of cells derived from breast cancer. The differences in effects of NRG-1 appear to depend on splice variation and interaction with receptor(s) (2). NRG-1 binds to ErbB3/HER3 or ErbB4/HER4. Binding induces dimerization with ErbB2/HER2. The Akt, Erk1/2 and Erk5 pathways have been shown to participate in NRG-1 activated signaling (4,5). NRG-1 appears to have roles in schizophrenia and breast cancer (1,4,6).

Background References:

- (1) Falls, D.L. (2003) *Exp Cell Res* 284, 14-30.
- (2) Chua, Y.L. et al. (2009) *Oncogene* 28, 4041-52.
- (3) Kalinowski, A. et al. (2010) *FASEB J*, [Epub ahead of print].
- (4) Montero, J.C. et al. (2008) *Clin Cancer Res* 14, 3237-41.
- (5) Grossmann, K.S. et al. (2009) *Proc Natl Acad Sci U S A* 106, 16704-9.
- (6) Mei, L. and Xiong, W.C. (2008) *Nat Rev Neurosci* 9, 437-52.

◀ The proliferation of MCF7 cells treated with increasing concentrations of hNRG-1 was assessed. After a 7 day treatment with hNRG-1 cells were incubated with a tetrazolium salt and the OD₄₅₀ - OD₄₉₀ was determined.