

Human Epidermal Growth Factor (hEGF)



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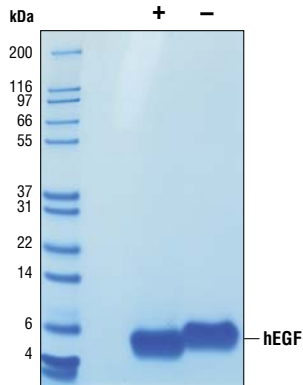
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Source: Recombinant human EGF (hEGF) Asn971-Arg1023 (Accession #NM_0011963) was produced in *E. coli* at Cell Signaling Technology.

Molecular Characterization: Recombinant hEGF has a Met on the amino terminus and has a calculated MW of 6353. DTT-reduced and non-reduced protein migrate as 6 kDa polypeptides. The expected amino-terminal MNSDS of recombinant hEGF was verified by amino acid sequencing.

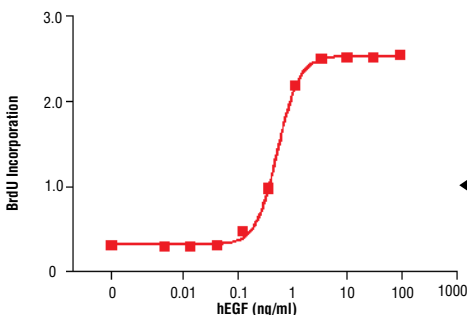
Endotoxin: Less than 0.01 ng endotoxin/1 μ g hEGF.

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

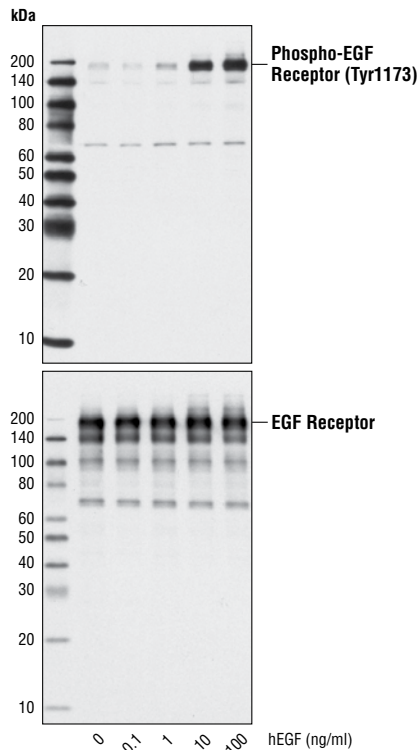


The purity of recombinant hEGF was determined by SDS-PAGE of 6 μ g reduced (+) and non-reduced (-) recombinant hEGF and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant hEGF was determined in a MCF10A cell proliferation assay. The ED₅₀ of each lot is between 10-600 pg/ml.



◀ The proliferation of MCF10A cells treated with increasing concentrations of hEGF was assessed. After 24 hr treatment, cells were labeled with BrdU for 4 hr. BrdU incorporation was determined by ELISA and the OD₄₅₀-OD₆₉₀ determined.



Western blot analysis of extracts from A-431 cells, untreated or treated with hEGF for 10 minutes, using Phospho-EGF Receptor (Tyr1173) (53A5) Rabbit mAb #4407 (upper) and EGF Receptor Antibody #2232 (lower).

Formulation: With carrier: Lyophilized from a 0.22 μ m filtered solution of PBS, pH 7.2 containing 20 μ g BSA per 1 μ g hEGF.

Carrier free: Lyophilized from a 0.22 μ m 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 hEGF concentration of greater than 50 μ g/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 hEGF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock hEGF should be greater than 50 μ g/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: EGF is produced by epithelial cells, fibroblasts and many other cell types (1,2). Low molecular weight soluble EGF is generated through proteolysis of a larger ~130,000 molecular weight transmembrane precursor (1,2). Both soluble and membrane forms of EGF are active (2). EGF induces proliferation, differentiation, and survival of many cell types including tumor-derived cells (1,2,3). There are multiple members of the EGF family and multiple members of the Erb/Her EGF receptor family. EGF binds to ErbB1/HER1 and induces homodimerization or induces heterodimerization with other Erb/Her members (1). Binding of EGF signals through the MAPK, PI3K/Akt, and STAT 5 pathways (1). EGF, EGF family members, EGF receptors and their signaling pathways are involved in many cancers and are targets for therapeutic intervention (1,2).

Background References:

- (1) Citri, A. and Yarden, Y. (2006) *Nat Rev Mol Cell Biol* 7, 505-16.
- (2) Higashiyama, S. et al. (2008) *Cancer Sci* 99, 214-20.
- (3) Xian, C.J. (2007) *Endocr Rev* 28, 284-96.