Recombinant Human EGF GMP

Catalog Number: 236-GMP

DESCRIPTION

Source	*E. coli*-derived human EGF protein Asn971-Arg1023, with an N-terminal Met Accession # P01133 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	Met-Asn$_{971}$-Ser-Asp-Ser-Glu-(Cys)-Pro-Leu-Ser
Predicted Molecular Mass	6 kDa

SPECIFICATIONS

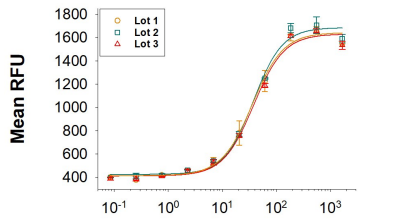
SDS-PAGE	6 kDa, reducing conditions
Activity	Measured in a cell proliferation assay using Balb/3T3 mouse embryonic fibroblast cells. Rubin, J.S. *et al.* (1991) Proc. Natl. Acad. Sci. USA **88**:415. The ED_{50} for this effect is 20-100 pg/mL. The specific activity of Recombinant Human EGF is >8.0 x 10^5 IU/mg, which is calibrated against the human EGF WHO International Standard (NIBSC code: 91/530).
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Mass Spectrometry	The result of the major peak from mass spectrometry analysis is 6347 Da, which corresponds to the calculated molecular mass of 6353 Da.
Host Cell Protein	<0.5 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative when tested in a ribosomal RNA hybridization assay.
Host Cell DNA	<0.0015 ng per µg of protein when tested by PCR.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 200 µg/mL in PBS.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, ≤ -20 °C under sterile conditions after reconstitution.

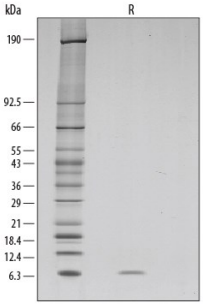
DATA

Bioactivity



GMP-grade Recombinant Human EGF (Catalog # 236-GMP) stimulates proliferation of the Balb/3T3 mouse embryonic fibroblast cell line. The ED_{50} for this effect is 20-100 pg/mL. Three independent lots were tested for activity and plotted on the same graph to show lot-to-lot consistency of GMP EGF.

SDS-PAGE



1 µg/lane of GMP-grade Recombinant Human EGF (Catalog # 236-GMP) was resolved by SDS-PAGE and visualized by silver staining under reducing (R) conditions, showing a single band at 6 kDa.

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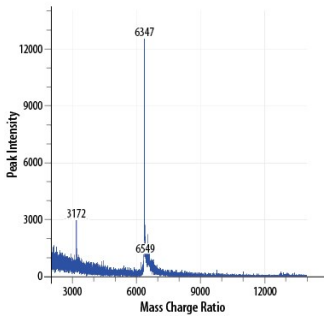
Mass Spectrometry



MALDI-TOF analysis of GMP-grade Recombinant Human EGF (Catalog # 236-GMP). The major peak corresponds to the calculated molecular mass, 6353 Da. The minor peak at 6549 Da is a matrix-associated artifact of the MALDI-TOF.

BACKGROUND

Epidermal growth factor (EGF) is a small, potent growth factor capable of inducing cell proliferation, differentiation, and survival. EGF is the founding member of the EGF family that also includes TGF-alpha, amphiregulin (AR), betacellulin (BTC), epiregulin (EPR), heparin-binding EGF-like growth factor (HB-EGF), epigen, and the neuregulins (NRG)-1 through -6 (1). Members of The EGF family are characterized by a shared structural motif, the EGF-like domain, which contains three intramolecular disulfide bonds that are formed by six similarly spaced, conserved cysteine residues (2). These disulfide bonds are essential for proper protein conformation and receptor binding. All EGF family members are synthesized as type I transmembrane precursor proteins that may contain several EGF domains in the extracellular region. The mature proteins are released from the cell surface by regulated proteolysis (1). The full length EGF protein is 1207 amino acids (aa) (EGF precursor) containing nine EGF domains and nine LDLR class B repeats. However, the mature protein is much smaller, only 53 aa, and is generated by proteolytic cleavage of the EGF domain proximal to the transmembrane region (3). EGF is well conserved across mammals with mature human EGF 70% identical to mature mouse and rat EGF. Physiologically, EGF is found in various body fluids, including blood, milk, urine, saliva, seminal fluid, pancreatic juice, cerebrospinal fluid, and amniotic fluid (4). EGF is a high affinity ligand of the EGF receptor (ErbB). Four ErbB (HER) family receptor tyrosine kinases including EGFR/ErbB1, ErbB2, ErbB3 and ErbB4, mediate responses to EGF family members (5). EGF binding induces dimerization of the EGF receptor resulting in activation of the protein tyrosine kinase signaling pathway. These receptors undergo a complex pattern of ligand-induced homo- or hetero-dimerization to transduce EGF family signals (6, 7). EGF binds ErbB1 and depending on the context, induces the formation of homodimers or heterodimers containing ErbB2. Dimerization results in autophosphorylation of the receptor at specific tyrosine residues to create docking sites for a variety of signaling molecules (5, 8). Biological activities ascribed to EGF include epithelial development, angiogenesis, inhibition of gastric acid secretion, fibroblast proliferation, and colony formation of epidermal cells in culture.

References:

1. Harris, R.C. *et al.* (2003) Exp. Cell Res. **284**:2.
2. Carpenter, G. and Cohen, S. (1990) J. Biol. Chem. **265**:7709.
3. Bell, G.I. *et al.* (1986) Nucl. Acids Res. **14**:8427.
4. Carpenter, G. and Zendegui, J.G. (1986) Exp. Cell Res. **164**:1.
5. Jorissen, R.N. *et al.* (2003) Exp. Cell Res. **284**:31.
6. Gamett, D.C. *et al.* (1997) J. Biol. Chem. **272**:12052.
7. Qian, X. *et al.* (1994) Proc. Natl. Acad. Sci. **91**:1500.
8. Qian, X. *et al.* (1999) J. Biol. Chem. **274**:574.

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- Mycoplasma testing by ribosomal RNA hybridization assay

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