

DESCRIPTION

Source	<i>E. coli</i> -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. <i>et al.</i> (1991) Proc. Natl. Acad. Sci. USA 88:415. The ED ₅₀ for this effect is 20-100 µg/mL. The specific activity of Recombinant Human EGF is approximately 1.4 x 10 ³ IU/µg, which is calibrated against 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.

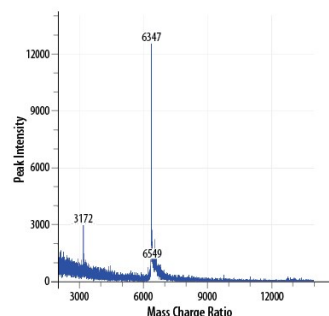
PREPARATION AND STORAGE

Reconstitution	Reconstitute at 200 µg/mL in PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> 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

<p>Bioactivity</p> <p>GMP-grade Recombinant Human EGF (Catalog # 236-GMP) stimulates proliferation of the Balb/3T3 mouse embryonic fibroblast cell line. The ED₅₀ for this effect is 20-100 µg/mL.</p>	<p>SDS-PAGE</p> <p>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.</p>
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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 the founding member of the EGF family that also includes TGF- α , 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 share a structural motif, the EGF-like domain, which is characterized by three intramolecular disulfide bonds that are formed by six similarly spaced conserved cysteine residues (2). 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 1207 amino acid (aa) human EGF precursor contains nine EGF domains and nine LDLR class B repeats. The mature protein consists of 53 aa and is generated by proteolytic excision of the EGF domain proximal to the transmembrane region (3). Mature human EGF shares 70% aa sequence identity with mature mouse and rat EGF. EGF is present in various body fluids, including blood, milk, urine, saliva, seminal fluid, pancreatic juice, cerebrospinal fluid, and amniotic fluid (4). Four ErbB (HER) family receptor tyrosine kinases including EGFR/ErbB1, ErbB2, ErbB3 and ErbB4, mediate responses to EGF family members (5). 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 Zendegei, 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.

MANUFACTURING SPECIFICATIONS

GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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- Equipment calibration schedules using a computerized calibration program
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- Material review process for variances
- Monitoring of stability over product shelf-life

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- N-terminal amino acid analysis, SDS-PAGE analysis, mass spectrometry results, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial bioburden testing (using broth culture, Sabourand's dextrose and blood agar plates with results reported at 3 days and at 7 days)
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

Additional testing and documentation requested by the customer can be arranged at an additional cost. Testing may include, but is not limited to, USP <61> bioburden testing, positive identity testing, testing for adventitious agents and testing for residual host cell content.

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- Dedicated fermentors are utilized in committed animal-free areas.

Purification

- Protein purification columns are animal-free.
- Bulk proteins are filtered using animal-free filters.
- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

Quality Assurance

- Low Endotoxin Level.
- No impairment of biological activity.
- High quality product obtained under stringent conditions.

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