

#### DESCRIPTION

**Source** *E. coli*-derived human EGF protein  
Asn971 - Arg1023, with an N-terminal Met  
Accession # P01133.2  
Produced using non-animal reagents in an animal-free laboratory.  
Manufactured and tested under cGMP guidelines.

**N-terminal Sequence Analysis** Met-Asn971-Ser-Asp-Ser-Glu-(Cys)-Pro-Leu-Ser

**Predicted Molecular Mass** 6 kDa

#### SPECIFICATIONS

**SDS-PAGE** 7 kDa, under 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<sub>50</sub> for this effect is 20.0-100 pg/mL.  
The specific activity of Recombinant Human EGF is >8.0 x 10<sup>5</sup> 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 with quantitative densitometry by Coomassie® Blue Staining.

**Mass Spectrometry** The molecular weight by mass spectrometry is 6343 Da ± 5 Da.

**Host Cell Protein** <0.500 ng per µg of protein when tested by ELISA.

**Mycoplasma** Negative for Mycoplasma.

**Host Cell DNA** <0.00150 ng per µg of protein when tested by PCR.

**Formulation** Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

#### PREPARATION AND STORAGE

**Reconstitution** Reconstitute at 500 µg/mL in sterile water.

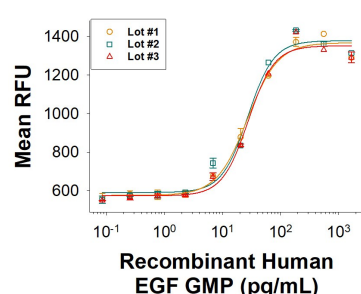
**Shipping** The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**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.

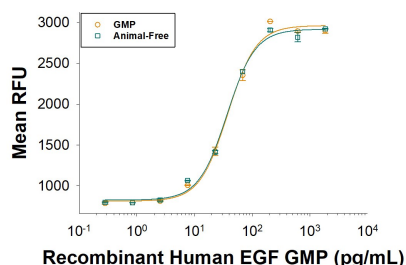
**DATA**

**Bioactivity**



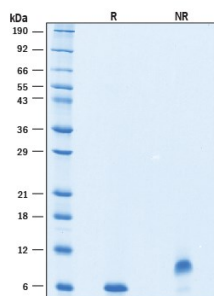
**Recombinant Human EGF GMP Protein Bioactivity.** The bioactivity of Recombinant Human EGF GMP Protein (Catalog # BT-EGF-GMP) was measured by its ability to stimulate the proliferation of the Balb/3T3 mouse embryonic fibroblast cell line. Three independent lots were tested for bioactivity and plotted on the same graph to show lot-to-lot consistency of GMP EGF protein.

**Bioactivity**



**Equivalent Bioactivity of GMP and Animal-Free grades of Recombinant Human EGF.** Equivalent bioactivity of GMP (Catalog # BT-EGF-GMP) and Animal-Free (Catalog # BT-EGF-AFL) grades of Recombinant Human EGF as measured in a cell proliferation assay using the cell proliferation of Balb/3T3 mouse embryonic fibroblast cell line (orange and green, respectively).

**SDS-PAGE**



**Recombinant Human EGF GMP Protein SDS-PAGE.** 2 µg/lane of Recombinant Human EGF GMP Protein (Catalog # BT-EGF-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at 7 kDa under reducing conditions.

**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.

## MANUFACTURING SPECIFICATIONS

### GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and Eu. Ph. 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

R&D Systems' quality focus includes:

- Designed, manufactured and tested under an ISO 9001:2015 and ISO 13485:2016 certified quality system
- Documented and controlled manufacturing process
- Control of documentation and process changes by QA
- Personnel training programs
- Raw material inspection and vendor qualification/monitoring program
- Validated equipment, processes and test methods
- Equipment calibration and maintenance schedules using a Regulatory Asset Manager
- Facility/Utilities maintenance, contamination controls, safety and pest control programs
- Material review process for variances
- Robust product stability program following relevant ICH guidelines

R&D Systems strives to provide our customers with the analytical characteristics of each product so that customers may determine whether our products are appropriate for their application. Each product is provided with a lot-specific Certificate of Analysis that contains the product's specifications and test results. Quality control testing may include, but is not limited to:

- N-terminal amino acid analysis
- SDS-PAGE purity analysis
- Molecular weight analysis via mass spectrometry
- Endotoxin assessment per USP <85> and Ph. Eur. 2.6.14 guidelines
- Bioassay analysis
- Microbial testing per USP <71> and Ph. Eur. 2.6.1 guidelines
- Host cell protein assessment
- Host cell DNA assessment
- Mycoplasma assessment

Additional testing and documentation requested by the customer can be arranged at an additional cost.

Production records and facilities are available for examination by appropriate personnel on-site at R&D Systems in Minneapolis and St. Paul, Minnesota USA.

R&D Systems sells GMP grade products for preclinical or clinical *ex vivo* use. They are not for *in vivo* use. Please read the following End User Terms prior to using this product.

### Animal-Free Manufacturing Conditions

Our dedicated controlled-access animal-free laboratories ensure that at no point in production are the products exposed to potential contamination by animal components or byproducts. Every stage of manufacturing is conducted in compliance with R&D Systems' stringent Standard Operating Procedures (SOPs). Production and purification procedures use equipment and media that are confirmed animal-free.

### Production

- All molecular biology procedures use animal-free media and dedicated labware.
- 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.

[Please read our complete Animal-Free Statement](#)

## PRODUCT SPECIFIC NOTICES

Full terms and conditions of sale can be found online in the Protein Sciences Segment T&Cs at: [Terms & Conditions](#).