Recombinant Human EGF

Catalog Number: 236-EG

DESCRIPTION

Source	*E. coli*-derived human EGF protein Asn971-Arg1023, with an N-terminal Met Accession # P01133
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	6 kDa

SPECIFICATIONS

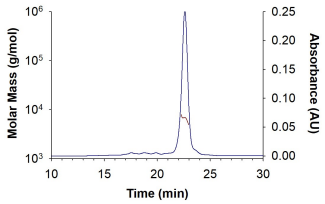
SDS-PAGE	6 kDa, reducing conditions
Activity	Measured in a cell proliferation assay using Balb/3T3 mouse embryonic fibroblast cells. Rubin, J.S. *et al.* (1991) Proc. Natl. Acad. Sci. USA **88**:415. The ED_{50} for this effect is 20-100 pg/mL.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 500 µg/mL in sterile 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.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

DATA

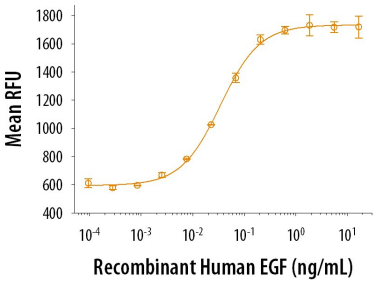
SEC-MALS



SEC-MALS Data	Result
Retention Time	22.2 - 23.0
MW - Predicted (Monomer)	6.0 kDa
MW - MALS	6.7 kDa
Polydispersity	1.008
System Suitability: BSA Monomer 66.4 ± 3.32 kDa	Pass

Recombinant Human EGF Protein SEC-MALS. Recombinant Human EGF Protein (Catalog # 236-EG) has a molecular weight (MW) of 6.7 kDa as analyzed by SEC-MALS, suggesting that this protein is a monomer.

Bioactivity



Recombinant Human EGF Protein Bioactivity Recombinant Human EGF (Catalog # 236-EG) stimulates cell proliferation of the Balb/3T3 mouse embryonic fibroblast cell line. The ED_{50} for this effect is 20-100 pg/mL.

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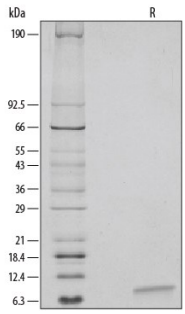
Recombinant Human EGF

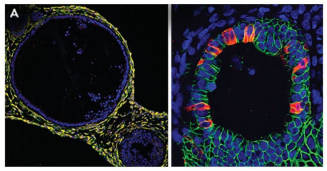
Catalog Number: 236-EG

SDS-PAGE



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

Cell Culture



iPSC-derived Human Intestinal Organoids Cultured using Recombinant Human EGF Protein. iPSC-derived human intestinal organoids were cultured using Cultrex™ UltiMatrix RGF Basement Membrane Extract (Catalog # BME001-05) and intestinal organoid culture medium, which includes Recombinant Human EGF (Catalog # 236-EG), Recombinant Human Noggin (Catalog # 6057-NG), Recombinant Human R-Spondin 1 (Catalog # 4645-RS), and Recombinant Human Wnt-3a (Catalog # 5036-WN), along with the other reagents listed in the intestinal organoid culture medium recipe in the human intestinal organoid culture protocol. (A) Human intestinal organoids were stained using a Rat Anti-Human/Mouse/Rat Vimentin Monoclonal Antibody (Catalog # MAB2105; green) and a Goat Anti-Human/Mouse Desmin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3844; red) to visualize myofibroblast cells and counterstained with DAPI (Catalog # 5748; blue). (B) Human intestinal organoids were stained using a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF748; green) and a Mouse Anti-Human MUC2 Monoclonal Antibody (Novus Biologicals, Catalog # NBP2-44431; red) and counterstained with DAPI (Catalog # 5748; blue).

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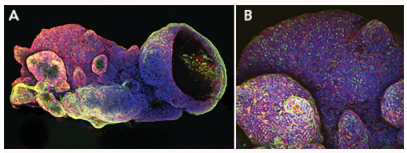
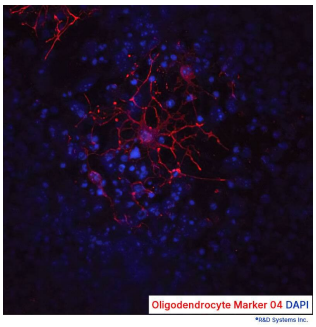
Cell Culture



Adult Stem Cell-derived Human Descending Colon Organoids Cultured using Recombinant Human EGF Protein. Adult stem cells isolated from human descending colon were embedded in Cultrex UltiMatrix RGF Basement Membrane Extract (Catalog # BME001-05) and cultured for 30 days in intestinal organoid culture medium, which includes Recombinant Human EGF (Catalog # 236-EG), Recombinant Human Noggin (Catalog # 6057-NG), Recombinant Human R-Spondin 1 (Catalog # 4645-RS), and Recombinant Human Wnt-3a (Catalog # 5036-WN), along with the other reagents listed in the intestinal organoid culture medium recipe in the human intestinal organoid culture protocol. (A) Organoids were fixed and stained with a Mouse Anti-Human MUC2 Monoclonal Antibody (Novus Biologicals; Catalog # NBP2-44431; green) to visualize intestinal goblet cells and counterstained with a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF748; red) and DAPI (Catalog # 5748; blue). The image shown was taken at 10x magnification. (B) Organoids were fixed and stained with a Mouse Anti-Human Chromogranin A Monoclonal Antibody (Catalog # MAB90981; green) to visualize enteroendocrine cells and counterstained with a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF748; red) and DAPI (Catalog # 5748; blue). The image shown was taken at 20x magnification.

Cell Culture



Culture and Characterization of Mouse Oligodendrocytes. D3 mouse embryonic stem cells were expanded in KO-ES Media supplemented with Bovine Fibronectin Protein (Catalog # 1030-FN) to support cell attachment and spreading, the ITS and N-2 Plus Media Supplements (Catalog # AR013 and Catalog # AR003), and a panel of growth factors for effective oligodendrocyte differentiation, including Recombinant Human FGF-basic, Recombinant Human EGF (Catalog # 236-EG), and Recombinant Human PDGF-AA (Catalog # 221-AA). Oligodendrocytes were detected using a Mouse Anti-Human/Mouse/Rat/Chicken Oligodendrocyte Marker O4 Monoclonal Antibody (Catalog # MAB1326). The cells were stained with the NorthernLights™-557 Affinity-purified Goat Anti-Mouse IgM Secondary Antibody (Catalog # NL019; red). The nuclei were counterstained with DAPI (Catalog # 5748;blue).

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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:

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