Sterile

#5331

Mouse Epidermal Growth Factor (mEGF)

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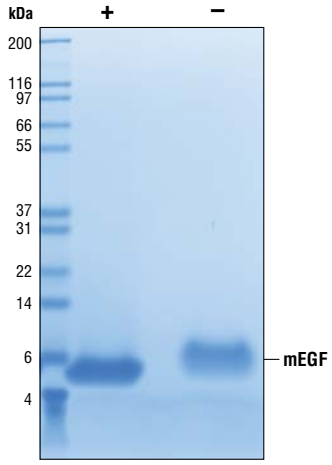
For Research Use Only. Not For Use In Diagnostic Procedures.

Source: Recombinant mouse EGF (mEGF) Asn977-Arg1029 (Accession #NP_034243) was produced in *E. coli* at Cell Signaling Technology.

Molecular Characterization: Recombinant mEGF has a Met on the amino terminus and has a calculated MW of 6045. DTT-reduced and non-reduced protein migrate as 6 kDa polypeptides. The expected amino-terminal MNSYP of recombinant mEGF was verified by amino acid sequencing.

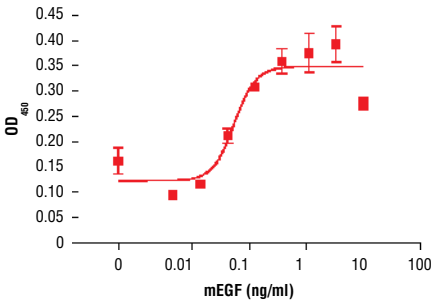
Endotoxin: Less than 0.01 ng endotoxin/1 µg mEGF.

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

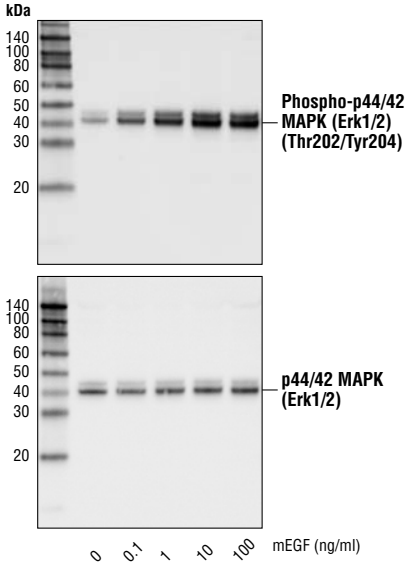


The purity of recombinant mEGF was determined by SDS-PAGE of 6 µg reduced (+) and non-reduced (-) recombinant mEGF and staining overnight with Coomassie Blue.

Bioactivity: The bioactivity of recombinant mEGF was determined in an NIH/3T3 cell proliferation assay. The ED_{50} of each lot is between 10-250 pg/ml.



The proliferation of NIH/3T3 cells treated with increasing concentrations of mEGF was assessed. After 24 hr treatment, cells were labeled with BrdU for 4 hr. BrdU incorporation was determined using the BrdU Cell Proliferation Assay Kit #6813 and the OD_{450} was determined.



Western blot analysis of extracts from NIH/3T3 cells, untreated or treated with mEGF for 10 minutes, using Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (D13.14.4E) XP® Rabbit mAb #4370 (upper) or p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb #4695 (lower).

Formulation: With carrier: Lyophilized from a 0.22 µm filtered solution of 20 mM citrate, pH 3.0 containing 100 mM NaCl and 20 µg BSA per 1 µg mEGF.

Carrier free: Lyophilized from a 0.22 µm filtered solution of 20 mM citrate, pH 3.0 containing 100 mM NaCl.

Reconstitution:
With carrier: Add sterile 20 mM citrate, pH 3.0 to a final mEGF concentration of greater than 50 µg/ml. Solubilize for 30 minutes at room temperature with occasional gentle vortexing.

Carrier free: Add sterile 20 mM citrate, pH 3.0 or 20 mM citrate, pH 3.0 containing protein to minimize absorption of mEGF to surfaces. Solubilize for 30 minutes at room temperature with occasional gentle vortexing. Stock mEGF should be greater than 50 µg/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 (1-3). There are multiple members of the EGF family and multiple members of the ErbB/HER EGF receptor family. EGF binds to ErbB1/HER1 and induces homodimerization or induces heterodimerization with other ErbB/HER members (1) Binding of EGF signals through the MAPK, PI3K/Akt, and Stat5 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.