Certificate of Analysis

Epidermal Growth Factor, Human, Recombinant (rhEGF):

Part No.	Size
G502A	100µg

Part# 9PIG502

Revised 4/18

AF9PIG502 0418G502

Description: Epidermal Growth Factor, Human Recombinant (rhEGF) is a 6.2kDa protein that is mitogenic for a variety of mammalian cell types (1–5). EGF stimulates the proliferation and differentiation of epithelial cells from skin, the cornea, lung and tracheal tissue and the gastrointestinal tract. EGF also promotes growth and migration of keratinocytes and enhances the proliferation of fibroblasts and embryonic cells. Thus, EGF plays an important role in wound healing and organogenesis. In addition to its proliferative effects, EGF demonstrates a variety of other bioactivities, including effects on cytoskeletal organization, cell migration and the synthesis and turnover of extracellular matrix molecules. Additionally, EGF promotes bone resorption by enhancing the release of calcium from bone tissue.

The biological effects of EGF are mediated by a specific transmembrane receptor. The EGF receptor is a 170kDa monomeric glycoprotein with intrinsic tyrosine kinase activity. Stimulation of the receptor kinase activity occurs when EGF binds to the extracellular domain of the receptor, resulting in autophosphorylation of the receptor's cytoplasmic tail and transduction of the EGF proliferative signal. The activated EGF receptor phosphorylates a number of cellular protein substrates, including phospholipase C-γ1 (PLC-γ1) and other proteins involved in signal transduction. Activation of PLC-γ1 results in the hydrolysis of inositol phospholipids and an increase in intracellular calcium levels, which subsequently activate protein kinase C (PKC). PKC activity, in turn, attenuates the tyrosine kinase activity of the EGF receptor (2). The proliferative response to EGF is further limited by rapid internalization and degradation of the ligand-receptor complex within lysosomes.

The EGF receptor is shared by several structurally related growth factors, including transforming growth factor-α (TGF-α) and EGF-like peptides produced by a number of viruses (e.g., vaccinia virus and Shope fibroma virus). A truncated version of the EGF receptor is produced from the viral oncogene v-*erb*-B. The deletion of the extracellular domain in the v-*erb*-B oncogene product results in uncontrolled EGF-independent signaling and proliferation.

EGF is commonly used as a supplement at 1–20ng/ml in serum-free or reduced serum media used for culture of mammalian cells. Human recombinant EGF is recommended for studies of the human EGF receptor.

Formulation: rhEGF is supplied as a dried powder and contains no additives.

Optimal Biological Range: The optimal rhEGF concentration in culture is 0.5–25ng/ml, depending on cell type.

Reconstitution: Reconstitute in culture medium, buffer, water or 0.1M acetic acid.

Source: Recombinant DNA expressed in *E. coli*.

Storage Conditions: Store desiccated at –20°C. See the expiration date on the product information label. Store reconstituted rhEGF in aliquots at –20°C, where it is stable for 3 months. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Quality Control Assays

Biological Activity: The ED_{50} for rhEGF, i.e., the concentration of factor that produces one-half the maximal response, is determined in a proliferation bioassay using BALB/c 3T3 fibroblasts in serum-free medium and the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Cat.# G5421). The ED_{50} value obtained is reported on the Product Information Label affixed to this document.

Specific Activity: An international reference standard 91/530 for recombinant EGF, provided by the National Institute for Biological Standards and Controls, is run in parallel with each batch of rhEGF and used to assign the specific activity.

References

1. Chicoine, M.R. and Silbergeld, D.L. (1997) Mitogens as motogens. *J. Neurooncol.* **35**, 249–57.
2. Yates, R.A. *et al.* (1991) Epidermal growth factor and related growth factors. *Int. J. Dermatol.* **30**, 687–94.
3. Jensen, R.L. (1998) Growth factor-mediated angiogenesis in the malignant progression of glial tumors: A review. *Surg. Neurol.* **49**, 189–95.
4. Morita, M. *et al.* (1996) Long-term survivors of glioblastoma multiforme: Clinical and molecular characteristics. *J. Neurooncol.* **27**, 259–66.
5. Groenen, L.C., Nice, E.C. and Burgess, A.W. (1994) Structure-function relationships for the EGF/TGF-α family of mitogens. *Growth Factors* **11**, 235–57.
6. Riss, T.L. *et al.* (1988) Human recombinant insulin-like growth factor I. I. Development of a serum-free medium for clonal density assay of growth factors using BALB/c 3T3 mouse embryo fibroblasts. *In Vitro Cell. Dev. Biol.* **24**, 1099–106.

Signed by:

R. Wheeler, Quality Assurance

Promega Corporation

2800 Woods Hollow Road
Madison, WI 53711-5399 USA
Telephone 608-274-4330
Toll Free 800-356-9526
Fax 608-277-2516
Internet www.promega.com

Part# 9PIG502
Printed in USA. Revised 4/18.

Promega

Usage Information

I. Sample Protocol to Determine Bioactivity of rhEGF with BALB/c 3T3 Fibroblasts

Promega uses the protocol supplied below to test the activity of rhEGF preparations. With appropriate modifications, this protocol can be used for cell proliferation assays in a variety of experimental applications.

Materials to Be Supplied by the User

(Solution compositions are provided in Section II.)

- CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Cat.# G5421)
- serum-free medium (SFM)
- ice-cold Dulbecco's PBS (DPBS)
- 0.05% (w/v) trypsin in DPBS (0.2µm filter-sterilized)
- 0.2% (w/v) soybean trypsin inhibitor (0.2µm filter-sterilized)
- F12/DME and 10% calf serum

A. Protocol

This protocol uses the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay to determine bioactivity of rhEGF. For a more detailed protocol for the CellTiter 96® AQ_{ueous} Assay, please request Technical Bulletin #TB169 (also available at: **www.promega.com**).

1. Three days prior to performing this assay, seed 1–2 × 10^5 BALB/3T3 clone A31 cells (ATCC CCL 163) per T-75 flask in F12/DME supplemented with 10% calf serum.
2. Pipet 50µl of SFM into each well of a 96-well plate.
3. For the negative control, use SFM with no addition of rhEGF in all the wells in column 1 of the 96-well plate.
4. Dilute rhEGF in SFM to a concentration that is 4 times the highest concentration to be assayed. Add 50µl of rhEGF diluted in SFM to column 12 in quadruplicate and perform 50µl serial dilutions across the plate to column 2. For rhEGF, the final concentration range should be 20ng/ml to 0.02ng/ml. Equilibrate the plates in a 37°C, 5% CO_2 humidified atmosphere while preparing the cell suspension.
5. Harvest BALB/c 3T3 clone A31 cells using the ice-cold trypsinization procedure (Section I.B).
6. Wash the cells in SFM by centrifugation at 300 × *g* at 4°C and count using a hemocytometer. Suspend the cells to 2 × 10^5 viable cells/ml in ice-cold SFM.
7. Add 50µl of the cell suspension (10^4 cells) to each well of the pre-equilibrated 96-well plate and return the plate to the incubator for 44 hours.
8. Add 20µl of fresh MTS/PMS solution, prepared as described in the *CellTiter 96® AQ_{ueous} Assay System Technical Bulletin* #TB169, into each well of the 96-well plate.
9. Incubate the plate at 37°C in a 5% CO_2, humidified atmosphere for 1–4 hours.
10. Record the absorbance at 490nm using an ELISA plate reader.
11. Plot the corrected absorbance at 490nm (Y-axis) versus concentration of rhEGF (X-axis). Figure 1 depicts the results of a typical assay using Promega materials. To determine the ED_{50} value, find the X-axis value that corresponds to one-half the difference between the maximum (plateau) and minimum (no rhEGF control) absorbance values.

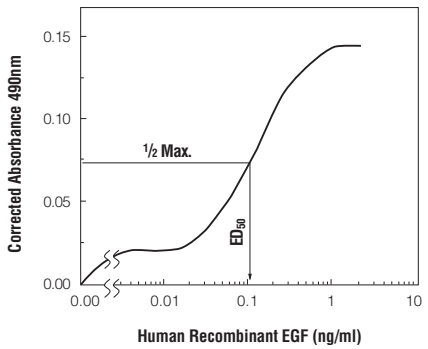

Figure 1. Proliferation of BALB/3T3 fibroblasts in response to various concentrations of rhEGF measured using the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay.

B. Ice-Cold Trypsinization Procedure (6)

1. Place crushed ice in a sealable plastic bag. Remove all the air from the bag by adding water or letting the ice melt to a slush consistency. Wipe the bag with 70% ethanol and place the bag in a laminar flow hood.
2. Place the flask of cells from the 37°C incubator directly on the bag of crushed ice and allow to cool for 3–4 minutes, and then remove the medium.
3. Rinse the flask with sterile, ice-cold DPBS.
4. Remove the DPBS and add 2ml of ice-cold 0.2µm filter-sterilized 0.05% (w/v) trypsin dissolved in DPBS. **Note:** Store stock solutions of trypsin at –20°C in 2ml aliquots. Thaw immediately before use and keep on ice.
5. After 1 minute, aspirate the trypsin solution and let the plate stand on the ice bag for an additional 3 minutes or until the cells round up and begin to detach when the flask is gently tapped.
6. Gently remove the monolayer of cells from the plastic surface by using a pipette to add 5ml of ice-cold 0.2µm filter-sterilized 0.2% (w/v) soybean trypsin inhibitor dissolved in DPBS. **Note:** Store aliquots of soybean trypsin inhibitor at –20°C and thaw immediately before use.
7. Transfer the solution to a 15ml sterile conical polypropylene centrifuge tube and bring the volume up to 10ml by adding SFM.
8. Centrifuge at 300 × *g* for 4 minutes at 4°C.
9. Aspirate the supernatant, gently suspend the cell pellet in 10ml of ice-cold SFM and centrifuge as in Step 8.
10. Aspirate the supernatant and gently resuspend the cell pellet in 10ml ice-cold SFM.
11. Using a hemocytometer, determine cell number and viability by counting trypan blue-treated aliquots of cells.

II. Composition of Buffers and Solutions

DPBS

0.2g/L	KCl
8.0g/L	NaCl
0.2g/L	KH_2PO_4
1.15g/L	Na_2HPO_4
133mg/L	$CaCl_2 \bullet 2H_2O$
100mg/L	$MgCl_2 \bullet 6H_2O$

SFM

Supplement F12/DME to contain a final concentration of:

10µg/ml	insulin
10µg/ml	transferrin
10ng/ml	selenium
100µg/ml	ovalbumin
1µM	dexamethasone
5µg/ml	fibronectin

F12/DME

Mix 1:1 (v:v) ratio of Ham's F12 and Dulbecco's modified Eagle's medium. Supplement to contain a final concentration of:

1.2g/L	sodium bicarbonate
15mM	HEPES

III. Related Products

Product	Size	Cat.#
EGF Receptor	10 units	V5551
CellTiter 96® AQ_{ueous} Non-Radioactive	1,000 assays	G5421
Cell Proliferation Assay	5,000 assays	G5430
	50,000 assays	G5440
CellTiter 96® AQ_{ueous} One Solution	200 assays	G3582
Cell Proliferation Assay	1,000 assays	G3580
	5,000 assays	G3581

For Reasearch Use Only Not for Use in Diagnostic Procedures.

Part# 9PIG502
Printed in USA. Revised 4/18.