Certificate of Analysis

Fibroblast Growth Factor, Basic, Human, Recombinant:

Part No.	Size
G507A	25µg

Part# 9PIG507

Revised 4/18

AF9PIG507 0418G507

Description: Fibroblast Growth Factor (FGF), Basic, also known as FGF-2, is a heparin binding growth factor that stimulates the proliferation of cells of mesenchymal, neuroectodermal and endothelial origin. Fibroblast Growth Factor, Basic, Human, Recombinant (rhFGF, Basic), is a 17.5kDa polypeptide containing 154 amino acids, that induces proliferation of multiple types of cells in vitro (1) and demonstrates potent angiogenic activity in vivo (2,3). Found in high concentrations in the brain, FGF, Basic, shares significant structural homology with FGF, Acidic (4), and elicits a similar target cell response, although FGF, Basic, is reported to possess greater biological activity (3). The amino acid sequence of this protein has been determined (3).

Target cells are vascular and corneal endothelial cells, mouse 3T3 fibroblasts, human prostate cells, chondrocytes, osteoblasts, myoblasts, smooth muscle and glial cells (3). rhFGF, Basic, is involved in activation of FGF receptor tyrosine kinase, proliferation of target cells and angiogenesis. For reviews on FGF, see references 5 and 6.

Formulation: rhFGF, Basic, is supplied as a dried powder.

Optimal Biological Range: For most in vitro applications rhFGF, Basic exerts its biological activity in the concentration range 0.1-10.0ng/ml.

Reconstitution: Dried rhFGF, Basic, is soluble in water and most aqueous buffers and can be diluted into other buffered solutions and stored at –20°C for future use.

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 rhFGF, Basic, in aliquots at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Quality Control Assays

Biological Activity: The ED_{50} for FGF, Basic, 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 Promega's 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: A basic fibroblast growth factor reference standard, #90/712, provided by the National Institute for Biological Standards and Controls, is run in parallel with each batch of rhFGF, Basic, and is used to assign the specific activity.

References

1. Bohlen, P. (1984) Isolation and partial molecular characterization of pituitary fibroblast growth factor. *Proc. Natl. Acad. Sci. USA* **81**, 5364.
2. Shing, Y. (1985) Angiogenesis is stimulated by a tumor-derived endothelial cell growth factor. *J. Cell. Biochem.* **29**, 275.
3. Esch, F. *et al.* (1985) Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine brain acidic FGF. *Proc. Natl. Acad. Sci. USA* **82**, 6507.
4. Esch, F. *et al.* (1985) Primary structure of bovine brain acidic fibroblast growth factor (FGF). *Biochem. Biophys. Res. Comm.* **133**, 554.
5. Jensen, R.L. (1998) Growth factor-mediated angiogenesis in the malignant progression of glial tumors: a review. *Surg. Neurol.* **49**, 189.
6. Webster, H.D. (1997) Growth factors and myelin regeneration in multiple sclerosis. *Multiple Sclerosis* **3**, 113.
7. 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.

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

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Promega

Usage Information

I. Sample Protocol to Determine Bioactivity of rhFGF, Basic, with BALB/c 3T3 Fibroblasts

Promega uses the protocol supplied below to test the activity of rhFGF, Basic, 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 Promega's CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay to determine bioactivity of rhFGF, Basic. For a more detailed protocol for the CellTiter 96® AQ_{ueous} Assay, please request Technical Bulletin #TB169 (also available on the Internet at www.promega.com).

1. Three days prior to performing this assay, seed 1–2 × 10^5 BALB/c 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 rhFGF, Basic, in all the wells in column 1 of the 96-well plate.
4. Dilute rhFGF, Basic, in SFM to a concentration that is 4 times the highest concentration to be assayed. Add 50µl of rhFGF, Basic, diluted in SFM to column 12 in quadruplicate and perform 50µl serial dilutions across the plate to column 2. For rhFGF, Basic, the final concentration range should be 50ng/ml to 0.05ng/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 10^5 viable cells/ml in ice-cold SFM.
7. Add 50µl of the cell suspension (containing 5,000 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 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 rhFGF, Basic, (X axis). 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 rhFGF, Basic, control) absorbance values.

B. Ice-Cold Trypsinization Procedure (7)

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	$CaCl_2 \cdot 2H_2O$
100mg/L	$MgCl_2 \cdot 6H_2O$

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 (Cat.# G5291)

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.#
CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay*	1,000 assays	G5421
	5,000 assays	G5430
	50,000 assays	G5440
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay*	200 assays	G3582
	1,000 assays	G3580
	5,000 assays	G3581

*For Research Use Only. Not for Use in Diagnostic Procedures.

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