

Product Contents

Insulin-Like Growth Factor-I, Human, Recombinant (rhIGF-I):

Part No.	Size
G511A	25µg

Description: Human Recombinant Insulin-Like Growth Factor-I (rhIGF-I) is a highly purified, biologically active, recombinant molecule, produced in yeast (*S. cerevisiae*). rhIGF-I is a 7.5kDa protein containing 70 amino acid residues, which stimulate the proliferation of a wide range of cell types including muscle, bone and cartilage tissue. IGFs control the biosynthesis of many intracellular and extracellular components (1,2) and are potent mitogens for mesenchymally derived cells (3–5). Growth hormone has been shown to mediate its effects on bone formation indirectly, through IGF-I (6,7). rhIGF-I is also known as Somatomedin-C.

Target cells for rhIGF-I are mammalian and avian fibroblasts (8,9), GH3 cells (10), chondrocytes (11), adipocytes (2), sertoli cells (12) granulosa cells and many other cell types of mesenchymal origin. rhIGF-I promotes activation of type I IGF receptor tyrosine kinase, sulfation of cartilage, insulin-like activity and proliferation of fibroblasts.

Formulation: rhIGF-I is supplied as a dried powder.

Reconstitution: The dried rhIGF-I powder should be rehydrated in 0.1M acetic acid. Recommended concentration for use is 1–10ng/ml.

Storage Conditions: Store desiccated at –20°C. See the expiration date on the product information label. Store reconstituted rhIGF-I in aliquots at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

Part# 9PIG511

Revised 5/05



Promega

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Quality Control Assays

Biological Activity: The ED₅₀ for rhIGF, 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 ED₅₀ value obtained is reported on the product information label affixed to this document.

Usage Information

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

The following protocol is used by Promega to test the activity of rhIGF-I 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
- 0.2% (w/v) soybean trypsin inhibitor
- F12/DME and 10% calf serum

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A. Protocol

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

- Three days prior to performing this assay, seed 1–2 x 10⁵ BALB/c 3T3 clone A31 cells per T-75 flask in F12/DME supplemented with 10% calf serum.
- Pipet 50µl of serum-free medium (SFM) into each well of a 96-well plate.
- As a negative control, add SFM **without** rhIGF-I to all wells (1–8) in column 1 of the 96-well plate.
- Dilute rhIGF-I in SFM to a concentration that is 4 times the highest concentration to be assayed. Add 50µl of rhIGF-I diluted in SFM to column 12 in quadruplicate and perform 50µl serial dilutions across the plate to column 2. For rhIGF-I, the final concentration range should be 80ng/ml to 0.08ng/ml. Equilibrate the plates in a 37°C, 5% CO₂ humidified atmosphere while preparing the cell suspension for addition to the wells.
- Harvest BALB/c 3T3 clone A31 cells (ATCC CCL 163) using the ice-cold trypsinization procedure (Section I.B).
- Wash the cells in SFM by centrifugation at 300 x g, 4°C and count the viable cells using a hemocytometer. Suspend the cells to 10⁵ cells/ml in ice-cold SFM.
- 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.
- Add 20µl of freshly prepared MTS/PMS solution into each well of the 96-well plate.
- Incubate the plate for 4 hours at 37°C in a 5% CO₂, humidified atmosphere.
- Record the absorbance at 490nm using an ELISA plate reader.
- Plot the corrected absorbance at 490nm (Y axis) versus concentration of rhIGF-I (X axis). To determine the ED₅₀ value, find the X-axis value that corresponds to one-half the difference between the maximum (plateau) and minimum (no IGF-I control) absorbance values.

B. Ice-Cold Trypsinization Procedure (13)

- 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-like consistency. Wipe the bag with 70% ethanol and place the bag in a laminar flow hood.
- 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, then remove the medium.
- Rinse the flask with sterile, ice-cold DPBS.
- 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 in 2ml aliquots at –20°C. Thaw immediately before use and keep on ice.
- 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.
- 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.
- Transfer the solution to a 15ml sterile conical polypropylene centrifuge tube and bring the volume up to 10ml by adding SFM.
- Centrifuge at 300 x g for 4 minutes at 4°C.
- Aspirate the supernatant, gently suspend the cell pellet in 10ml of ice-cold SFM and centrifuge as in Step 8.
- Aspirate the supernatant and gently resuspend the cell pellet in 10ml ice-cold SFM.
- 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	NaHPO ₄
1.15g/L	NaH ₂ PO ₄

F12/DME

Add 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

SFM

Supplement F12/DME to contain a final concentration of:

5µg/ml	fibronectin
5ng/ml	rhFGF, Basic (Cat.# G5071)
10µg/ml	transferrin
100µg/ml	ovalbumin
1µM	dexamethasone

III. Related Products

Product	Size	Cat.#
CellTiter 96® AQ _{ueous} One Solution Cell Proliferation Assay	200 assays	G3582
	1,000 assays	G3580
	5,000 assays	G3581
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} MTS Reagent Powder	1g	G1111
	250mg	G1112
CellTiter 96® Non-Radioactive Cell Proliferation Assay	1,000 assays	G4000
	5,000 assays	G4100

For Laboratory Use.

IV. References

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