

## Product Contents

### Interleukin-4, Human, Recombinant:

Part No.	Size
G559A	5µg

**Description:** Human Recombinant Interleukin-4 (rhIL-4) is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells. rhIL-4 is a 14kDa protein containing 130 amino acid residues.

Target cells for IL-4 include macrophages, myeloid progenitors, mast cells, T cells and B cells. The biological effects include B cell expression of MHC Class II molecules and immunoglobulins, and growth of T cells and mast cells. No mouse-human cross-species activity is found.

**Formulation:** rhIL-4 is supplied as a dried powder.

**Optimal Biological Range:** For most *in vitro* applications, rhIL-4 exerts its biological activity in the concentration range of 0.01–10ng/ml.

**Reconstitution:** The dried rhIL-4 is soluble in water and most aqueous buffers, and can be reconstituted in PBS to a concentration of 100ng/ml. This solution can be diluted into other buffered solutions or stored at –20°C for up to 3 months.

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

**Storage Conditions:** Store desiccated below –20°C. See the expiration date on the product information label. Store reconstituted rhIL-4 in aliquots at –20°C, where it is stable for up to 3 months. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes.

### Quality Control Assays

**Biological Activity:** The ED<sub>50</sub> for rhIL-4, i.e., the concentration of factor that produces one-half the maximal response, is determined in a proliferation bioassay using TF-1 cells and Promega's CellTiter 96® AQ<sub>ueous</sub> Non-Radioactive Cell Proliferation Assay (Cat.# G5421). The ED<sub>50</sub> value obtained is reported on the Product Information Label affixed to this document.

**Specific Activity:** The specific activity of rhIL-4 is assigned by direct comparison with a Reference Standard from the National Institute for Biological Standards and Controls.

Part# 9PIG559

Revised 5/05



AF9PI G559 0505G559



**Promega**

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## I. Sample Protocol to Determine Bioactivity of rhIL-4 with TF-1 Cells

Promega uses the protocol supplied below to test the activity of rhIL-4 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

- CellTiter 96® AQ<sub>ueous</sub> Non-Radioactive Cell Proliferation Assay (Cat.# G5421)
- Complete medium (RPMI 1640 medium supplemented to contain a final concentration of 10% fetal bovine serum).
- Human recombinant GM-CSF (PeproTech Cat.# 300-03)

### A. Protocol

This protocol uses the CellTiter 96® AQ<sub>ueous</sub> Non-Radioactive Cell Proliferation Assay to determine bioactivity of rhIL-4. For a more detailed protocol for the CellTiter 96® Assay, please request Technical Bulletin #TB169.

- Maintain cultures of TF-1 cells in upright T-75 flasks in complete medium with 2ng/ml Human Recombinant GM-CSF. Subculture and re-feed cells with GM-CSF every 2–3 days or when they reach a density of  $5 \times 10^5$ /ml. Seed the cells at  $1 \times 10^5$ /ml.
- Subculture the TF-1 cells 2 days prior to the assay.
- Pipet 50µl of complete medium into each well of a 96-well tissue culture plate.  
**Note:** Do not include GM-CSF in the complete media used for the assay.
- Use wells in column 1 containing complete medium with no addition of rhIL-4 for the negative control.
- Dilute rhIL-4 in complete medium to a concentration that is 4 times the highest concentration to be assayed. Add 50µl of the rhIL-4 diluted in complete medium to column 12 in quadruplicate and perform 50µl serial dilutions across the plate to column 2. For Human Recombinant IL-4, the final concentration range should be 40ng/ml to 0.04ng/ml. Equilibrate the plate at 37°C, 5% CO<sub>2</sub> in a humidified atmosphere while preparing the cell suspension for addition to the wells.
- Harvest the TF-1 cells. Wash the cells twice in complete medium by centrifugation at  $300 \times g$  for 4 minutes.
- Determine the cell number and trypan blue viability. Suspend the cells to a final concentration of  $4 \times 10^5$ /ml in complete medium.
- Add 50µl of the cell suspension (containing 20,000 cells) to each well of the 96-well plate and return the plate to the incubator for 48 hours.
- Add 20µl of freshly prepared MTS/PMS solution from the CellTiter 96® AQ<sub>ueous</sub> Assay into each well of the 96-well plate.
- Incubate the plate at 37°C in a humidified, 5% CO<sub>2</sub> atmosphere for 1–4 hours.
- Record the absorbance at 490nm using an ELISA plate reader.
- Plot the corrected absorbance at 490nm (Y axis) versus concentration of rhGM-CSF (X axis). To determine the ED<sub>50</sub> value, find the X-axis value that corresponds to one-half the difference between the maximum (plateau) and minimum (no rhIL-4 control) absorbance values.

## II. Related Products

Product	Size	Cat.#
rhIL-1β	3µg	G5511
rhIL-6	25µg	G5541
CellTiter 96® AQ <sub>ueous</sub> Non-Radioactive Cell Proliferation Assay*	1,000 assays	G5421
	5,000 assays	G5430
	50,000 assays	G5440
CellTiter 96® AQ <sub>ueous</sub> One Solution Cell Proliferation Assay*	200 assays	G3582
	1,000 assays	G3580
	5,000 assays	G3581
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
	10 × 10ml	G7571
	100ml	G7572
	10 × 100ml	G7573

\*For Laboratory Use.