Certificate of Analysis

Tumor Necrosis Factor-α, Human, Recombinant:

Part No.	Size
G524A	10µg

Part# 9PIG524

Revised 7/13

Description: Tumor Necrosis Factor-α, Human, Recombinant (rhTNF-α) is a 17kDa protein containing 157 amino acid residues. Biological effects of this molecule include induction of apoptosis, cytolysis or cytostasis of tumor cells, activation of polymorphonuclear leukocytes, antiviral activity and induction of IL-1 or colony stimulating factor expression. rhTNF-α is supplied as a dried powder and contains no additives.

TNF-α is a pleiotropic cytokine produced predominantly by activated monocytes/macrophages. TNF-α is a primary mediator of numerous immunologic functions, including hemorrhagic tumor necrosis/cytotoxicity, inflammation and regulation of antiviral and immune proliferative and activation responses. As a central player in the cytokine network, TNF-α has been implicated in a variety of disease states, including cachexia, endotoxic (septic) shock, acute respiratory distress syndrome and a number of necrotic, proliferative and autoimmune diseases. TNF-α exists in both a secreted and a membrane bound form. References 1 and 2 are TNF-α reviews.

The diverse range of biological activities of TNF-α are mediated by two distinct receptors, TNF-R1 and TNF-R2, with approximate molecular weights of 55 and 75kDa, respectively. A recent model of receptor-mediated signaling (3) proposes that TNF-R1 mediates TNF-α signals associated with cytotoxicity, antiviral activity, fibroblast proliferation and the induction of NF-κB, and TNF-R2 mediates signals associated with proliferation of primary thymocytes and cytotoxic T cell lines. Signaling is thought to occur when trimeric TNF-α binds and aggregates receptor monomers. Soluble forms of the TNF receptors have been identified; these are derived from membrane-bound TNF receptors by proteolytic cleavage and appear to inhibit TNF activity in vivo (2). A structurally and functionally similar cytokine, TNF-β (lymphotoxin) binds both the 55kDa and 75kDa receptors (4). rhTNF-α is species cross-reactive.

Optimal Biological Range: TNF-α exerts its biological activity in the range of 0.05–20ng/ml.

Reconstitution: Reconstitute in sterile water to a final concentration of 100ng/µl. This solution can be further diluted into other solutions or buffers containing carrier protein.

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

Storage Conditions: Store powder desiccated at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. Store reconstituted TNF-α at –20°C for up to three months. See the expiration date on the Product Information Label.

Quality Control Assays

Biological Activity: The IC_{50} value (i.e., the concentration of cytotoxic agent necessary to decrease cell growth by 50%) for rhTNF-α is determined in a bioassay using murine L929 cells and the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Cat.# G5421). The IC_{50} value obtained is reported on the Product Information Label affixed to this document.

Specific Activity: An interim TNF-α reference standard, #87/650, provided by the National Institute for Biological Standards and Controls, is run in parallel with each batch of rhTNF-α and used to assign the specific activity.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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Promega

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# 9PIG524
Printed in USA. Revised 7/13

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Usage Information

1. Sample Protocol to Determine Bioactivity of rhTNF-α Using L929 Cells

The following protocol is used by Promega to test the activity of rhTNF-α preparations. With appropriate modifications, this protocol can be used for cytotoxicity assays in a variety of experimental applications. rhTNF-α exhibits an IC_{50} value below 0.1ng/ml when measured with the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay using murine L929 fibroblasts. (IC_{50} = the concentration of cytotoxic agent necessary to decrease cell growth by 50%.)

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)
- trypsin:EDTA
- complete medium
- horse serum
- actinomycin D
- L929 cells (ATCC# CCL 1)

A. Protocol

This protocol uses the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay to determine bioactivity of rhTNF-α. A more detailed protocol for the CellTiter 96® AQ_{ueous} Assay is available in Technical Bulletin #TB169.

1. Grow murine L929 cells in complete medium.
2. Harvest cells in log phase growth using trypsin:EDTA. Determine cell number and viability by trypan blue exclusion.
3. Dispense 90µl of the cell suspension (2×10^4 cells) into each well of a 96-well plate.
4. Incubate overnight (18–24 hours) at 37°C in a 5% CO_2, humidified atmosphere.
5. Prepare twofold serial dilutions of TNF-α in complete medium containing 10µg/ml actinomycin D. Add 10µl per well of these dilutions to yield a final concentration of 1µg/ml actinomycin D in all wells. The final TNF-α concentrations in the assay wells should range from 0.78ng/ml to 1.5pg/ml. The positive control should contain 200ng/ml TNF-α and 1µg/ml actinomycin D. The negative control should contain no TNF-α
6. Incubate the plate for 20 hours at 37°C in a 5% CO_2, humidified atmosphere.
7. Add 20µl of freshly prepared MTS/PMS solution to each well of the 96-well plate.
8. Incubate the plate for 4 hours at 37°C in a 5% CO_2, humidified atmosphere.
9. Record the absorbance at 490nm using an ELISA plate reader.
10. Plot the absorbance at 490nm (Y axis) versus concentration of TNF-α (X axis) (see Figure 1).

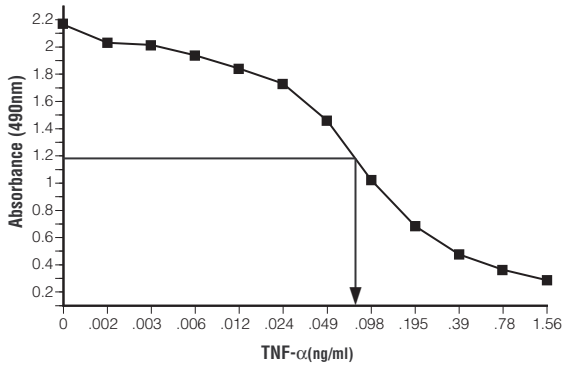

Figure 1. Killing of murine L929 cells in response to rhTNF-α, measured using the CellTiter96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay.

2. Composition of Buffers and Solutions

complete medium

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
10%	horse serum

trypsin:EDTA

Per liter of Hanks' balanced salt solution:

0.5g	porcine trypsin
0.2g	EDTA

Hanks' balanced salt solution (HBSS)

0.4g/L	KCl
0.06g/L	K_2HPO_4
8.0g/L	NaCl
0.048g/L	NaH_2PO_4
1.0g/L	D-glucose
0.35g/L	sodium bicarbonate

Final pH should be 6.7.

3. Related Products

Product	Size	Cat.#
CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay*	200 assays	G3582
CellTiter-Glo® Luminescent Cell Viability Assay	10ml	G7570
Caspase-Glo® 3/7 Assay	2.5ml	G8090
Caspase-Glo® 8 Assay	2.5ml	G8200
Caspase-Glo® 9 Assay	2.5ml	G8210
MultiTox-Fluor Multiplex Cytotoxicity Assay	10ml	G9200

Available in additional sizes.

IV. References

1. Arvin, B. *et al.* (1996) The role of inflammation and cytokines in brain injury. *Neurosci. Biobehav. Res.* **20**, 445–52.
2. Chicoine, M.R. and Silbergeld, D.L. (1997) Mitogens as motogens. *J. Neurooncol.* **35,** 249–57.
3. Tartaglia, L.A. and Goeddel, D.V. (1992) Two TNF receptors. *Immunol. Today* **13**, 151–3.
4. Ruddle, N.H. (1992) Tumor necrosis factor (TNF-alpha) and lymphotoxin (TNF-beta). *Curr. Opin. Immunol.* **4** , 327–32.

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Printed in USA. Revised 7/13