For life science research only.
Not for use in diagnostic procedures.

Tumor Necrosis Factor-α, human (hTNF-α)

recombinant (*E. coli*)

Version: 17
Content Version: May 2021

Cat. No. 11 371 843 001 1,000,000 U
10 µg, 1 ml

Store product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	Tumor Necrosis Factor-α, human (hTNF-α)	▪ Solution, filtered through 0.2 µm pore size membrane. ▪ 10 µg/ml solution in PBS (phosphate buffered saline) and 1 mg/ml BSA (bovine serum albumin). *Purity of BSA: >98%, endotoxin (LAL): <1 EU/mg BSA.*	1 bottle, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

The product is shipped on dry ice.
When stored at −15 to −25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	red	Tumor Necrosis Factor-α, human (hTNF-α)	Store in aliquots at −15 to −25°C. ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- 96 well tissue-culture grade, flat-bottomed microplates
- CO_2 incubator
- Centrifuge
- ELISA reader

For the quantitative determination of cytotoxic activity of human TNF-α

- Culture medium, such as RPMI 1640, containing 10% FCS (fetal calf serum), and 2 M glutamine.
- Actinomycin D, 5 mg/ml stock solution in water or ethanol (filter through 0.2 µm filter before use).
- TNF-α, human, recombinant, 10 µg/ml solution.
- MTT (3′-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) stock solution: 5 mg/ml in PBS.
- SDS/HCl stock solution: 15% SDS in 15 mM HCl.

1.4. Application

This product is intended for use in life sciences research applications only.

- hTNF-α has activating and growth stimulating activities on a variety of normal cells and has antiviral enhancing activity on many cell types *in vitro*, see table below.
- Causes selective necrosis of murine tumors when injected into tumor-bearing mice.
- *In vitro*, hTNF-α has direct cytolytic or cytostatic activity on certain transformed cells. In this context, it acts synergistically with interferon-γ.
- Cytotoxicity of hTNF-α for tumor cells has been known since the initial description of this factor.
- hTNF-α has important effects on several types of normal cells and may have profound effects on inflammatory reactions, bone resorption, the development and function of granulocytes, hemostasis, and lipid metabolism; in this context, hTNF-α is also known as cachectin.

Response of various cell lines to TNF-α *in vitro*

Response	Cell Line
Growth Enhancement	CCD-18Co (normal human colon) Detroit 551 (normal human fetal skin) FS-4 (normal human foreskin) FS-48 (normal human foreskin) LL24 (normal human lung) NRK-49F (normal rat kidney) Osteoclasts WI138 (normal human fetal lung) WI-1003 (normal human lung)
Null response	A549 (human lung carcinoma) B16 (murine melanoma) B16F10 (murine melanoma) Calu-3 (human lung carcinoma) CMT-93 (murine rectal carcinoma) G-361 (human melanoma) HeLa (human cervical carcinoma) HeLa D98 (human cervical carcinoma) HT-29 (human colon carcinoma) HT1080 (human fibrosarcoma) KB (human oral epidermoid carcinoma) LS174T (human colon carcinoma) RD (human rhabdosarcoma) Saos-2 (human osteogenic sarcoma) SK-CO-1 (human colon carcinoma) SK-LU-1 (human lung carcinoma) SK-OV-3 (human ovarian carcinoma) SK-UT-1 (human uterine carcinoma) S49 (murine lymphoma) T24 (human bladder carcinoma) WI138 VA 13 (human transformed W138)
Antiproliferative response	BT-20 (human breast carcinoma) BT-475 (human breast carcinoma) B6MS2 (murine sarcoma) B6MS5 (murine sarcoma) CMS4 (murine sarcoma) CMS16 (murine sarcoma) L929 (murine fibroblast) MCF7 (human breast carcinoma) ME-180 (human cervical carcinoma) Meth A (murine sarcoma) MMT (murine breast carcinoma) SAC (Moloney-transformed murine 3T3) SK-MEL-109 (human melanoma) SK-OV-4 (human ovarian carcinoma) UV1591-RE (murine fibrosarcoma) WEHI-164 (murine sarcoma) WiDr (human colon carcinoma)

2. How to Use this Product

2.1. Before you Begin

General Considerations

Primary structure

One polypeptide chain (158 amino acids) is identical to natural hTNF-α (157 amino acids) but with an extra methionine at the amino-terminus.

Working Solution

Dilute the concentrated hTNF-α solution (10 µg/ml) with PBS or culture medium containing BSA or HSA (human serum albumin), 1 mg/ml (0.1%) or 1 to 10% serum.

2.2. Protocols

Quantitative determination of cytotoxic activity of human TNF-α on sensitive cells

The following steps were performed using WEHI 164 cells.

1. Prepare hTNF-α test samples as serial dilutions (2 fold steps) in culture medium on a 96-well, flat-bottomed microplate in a final volume of 50 µl (range, for example, from 0.01 pg/ml to 100 pg/ml).

2. Adjust sensitive cells, such as WEHI 164 cells to 1.0 × 10^6 cells/ml in culture medium containing 1 µg/ml actinomycin D.
   – Incubate 3 hours at +37°C and 5% CO_2.

3. Spin cells down and resuspend to a final concentration of 1.0 × 10^6 cells/ml in culture medium containing 2 µg/ml actinomycin D.

4. Add 50 µl of this cell suspension to 50 µl of the prediluted hTNF-α samples in each well of the microplate.
   Final cell concentration: 5 × 10^5 cells/ml (5 × 10^4 cells/well).

5. Incubate the microplate overnight at +37°C and 5% CO_2.

6. After the incubation period, add 10 µl MTT solution (5 mg/ml) to each well and incubate for 4 hours at +37°C and 5% CO_2.

7. Terminate the reaction by adding 100 µl SDS/HCl solution (15% SDS in 15 mM HCl) into each well.
   – Incubate overnight at +37°C and 5% CO_2 to dissolve the blue formazan crystals and bleach the phenol red color of the cultures.

8. Evaluate the microplate on a microplate ELISA reader by using 550 nm and 690 nm as test and reference wavelength, respectively, see section **Results, Figure 1**.

The results depend strongly on the experimental setup, including cell line, especially the type of substrain, and culture conditions, such as cell density, incubation period, and actinomycin D concentration.

2.3. Parameters

Molecular Weight

17,000 Da

Purity

≥95% pure as determined by SDS-PAGE.
Endotoxin level: ≤10 EU/ml (LAL).
1 EU corresponds to 0.1 ng.

Specific Activity

≥100 MU/mg
Cell lytic assay with WEHI 164 cells (mouse fibrosarcoma cells) in the presence of actinomycin D (NIBSC interim standard, 87/650), see section **Results, Figure 1**.
The biological activity of this product may vary in different in vitro applications. Determine the optimal concentration range for specific applications.

Specificity

Human TNF-α is effective on mouse and human cells.

Unit Definition

EC_{50} definition

The amount of hTNF-α that is required to mediate half-maximal cytotoxicity (MTT cleavage) with WEHI 164 cells in the presence of actinomycin D (1 unit equals ≤0.01 ng/ml).

Working Concentration

For total cell lysis of sensitive cell lines, use approximately 1 ng/ml.

3. Results

Cytotoxic activity of recombinant hTNF-α

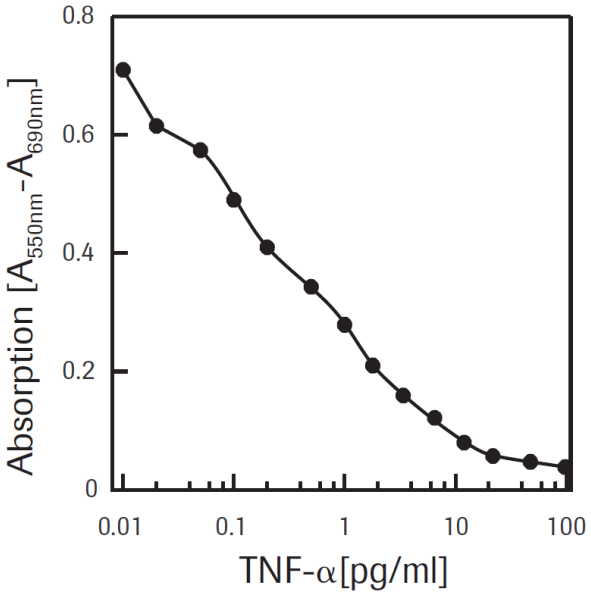

Fig. 1: Determination of the cytotoxic activity of recombinant hTNF-α on WEHI-164 cells using the described procedure.

4. Additional Information on this Product

4.1. Test Principle

hTNF-α is produced by activated monocytes and macrophages. It has been highly purified and found to have a molecular weight of 17,000 Da (SDS-PAGE). Under nondenaturing conditions, human TNF-α has a molecular weight of approximately 45,000 Da, suggesting that the native protein associates in an oligomeric form.

Preparation

Recombinant Tumor Necrosis Factor-α (hTNF-α) is produced in *E. coli* and purified by standard chromatographic techniques.

4.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Cell Proliferation Kit I (MTT)	1 kit, 2,500 tests	11 465 007 001

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

5.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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