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

recombinant (E. coli) solution, through a 0.2 μm pore size membrane filtered

Cat. No. 11 371 843 001
10 μg (1 ml)

Introduction

Tumor Necrosis Factor-α (hTNF-α) shows cytolytic and/or cytostatic activity on a variety of transformed cell lines. hTNF-α has also activating and growth stimulating activities on a variety of normal cells and has antiviral enhancing activity on many cell types in vitro (see table). It has been reported in research studies that in vivo hTNF-α can cause the necrosis of certain tumors.

Preparation

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

Primary structure

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

Molecular weight

17,000

Purity

Recombinant hTNF-α is > 95% pure as determined by SDS-PAGE.

Specific activity/EC₅₀

Specific activity is > 1.0 × 10⁸ U/mg [cell lytic assay with WEHI 164 cells (mouse fibrosarcoma cells) in the presence of actinomycin D] (see fig). (NIBSC interim standard, 07/650) (11,12).

EC₅₀ definition/Unit definition

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

Species specificity

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

Working concentration

For complete cell lysis of sensitive cell lines about 1 ng/ml is recommended.

Formulation

10 μg/ml in PBS and BSA, 1 mg/ml; (purity of BSA > 98%, endotoxin (LAL-test) < 0.1 EU/μg BSA); through a 0.2 μm pore size membrane filtered.

Endotoxin level

< 0.1 EU/μg (LAL-test).

Stability

Stable at −15 to −25°C. It is recommended to store the solution in aliquots at −15 to −25°C. Repeated freezing and thawing should be avoided.

Recommended method of dilution

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

Application

This product is intended for use in life sciences research applications only. Human TNF-α is produced by activated monocytes and macrophages. Human TNF-α has been highly purified and found to have a molecular weight of 17,000 (SDS-PAGE). Under non-denaturing conditions, hTNF-α has a molecular weight of approximately 45,000, suggesting that the native protein associates in an oligomeric form.

hTNF-α 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 (12-15). In this context it acts synergistically with interferon-γ (16, 17). Cytotoxicity of hTNF-α for tumor cells has been reported since the initial description of this factor. It has been recognized that hTNF-α also 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) (10, 18-20). It has also potent antiviral activity in vitro (21, 22).

Growth Enhancement

|--------------------|-------------------------------|--------------------------------------|-----------------------------|-------------------------------|---------------------------|---------------------------|--------------------------------|---------------------------------|

Null response

|----------------|-----------------------------|------------------------|--------------------------|-------------------------------|-------------------------------|--------------------------|-----------------------------|--------------------------------|---------------------------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|--------------------------------|-----------------------------|--------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
The results depend strongly on the experimental setup including cell line (especially the type of substrain) and culture conditions (e.g., cell density, incubation period, actinomycin C1 concentration).

Antiproliferative response

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Cell Line</th>
</tr>
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<tbody>
<tr>
<td>BT-20</td>
<td>(human breast carcinoma)</td>
</tr>
<tr>
<td>BT-475</td>
<td>(human breast carcinoma)</td>
</tr>
<tr>
<td>B0M52</td>
<td>(murine sarcoma)</td>
</tr>
<tr>
<td>B6M55</td>
<td>(murine sarcoma)</td>
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<tr>
<td>CMS4</td>
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<tr>
<td>MCF7</td>
<td>(human breast carcinoma)</td>
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<tr>
<td>ME-180</td>
<td>(human cervical carcinoma)</td>
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<tr>
<td>MethA</td>
<td>(murine sarcoma)</td>
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<tr>
<td>MMT</td>
<td>(murine breast carcinoma)</td>
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<tr>
<td>SAC</td>
<td>(Meloney-transformed murine 3T3)</td>
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<td>(human melanoma)</td>
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<td>SK-OV-4</td>
<td>(human ovarian carcinoma)</td>
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<tr>
<td>UV1591-RE</td>
<td>(murine fibrosarcoma)</td>
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<tr>
<td>WEHI-164</td>
<td>(murine sarcoma)</td>
</tr>
<tr>
<td>WI-Dr</td>
<td>(human colon carcinoma)</td>
</tr>
</tbody>
</table>

Tab.: Response of various cell lines to hTNF-α or TNF-β in vitro (10).

Working instruction for the quantitative determination of cytotoxic activity of hTNF-α on sensitive cells (exemplary with WEHI-164-cells).

Solutions

- Culture medium, e.g., RPMI 1640 containing FCS (fetal calf serum), 10% and 2 M glutamine.
- Actinomycin C, (Actinomycin D), 5 mg/ml stock solution in water or ethanol (filtered through a 0.2 μm pore size membrane before use).
- hTNF-α, human, rec., 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.

Procedure

- 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 e.g., from 0.01 pg/ml to 100 pg/ml; see fig.).
- Adjust sensitive cells, e.g., WEHI 164 cells, to 1.0 × 10⁶ cells/ml in culture medium containing actinomycin C1, 1 μg/ml and incubate 3 h at +37°C and 5% CO₂.
- Spin cells down and resuspend to a concentration of 1.0 × 10⁶ cells/ml in culture medium containing actinomycin C1, 2 μg/ml.
- 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 × 10⁵ cells/ml, i.e. 5 × 10⁴ cells/well).
- Incubate the microplate over night at +37°C and 5% CO₂.
- After the incubation period add 10 μl MTT-solution (5 mg/ml), to each well of the microplate and incubate for 4 h at +37°C and 5% CO₂.
- Terminate the reaction by adding 100 μl SDS/HCl solution (15% SDS in 15 mM HCl), into each well of the microplate and incubate overnight at +37°C and 5% CO₂ to dissolve the blue formazan crystals and bleach the phenol red color of the cultures.
- Evaluate the microplate on a microplate ELISA-reader by using 550 nm and 690 nm as test and reference wavelength, respectively.

Note

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Changes to
Previous Version

Editorial Changes

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Regulatory
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References