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1. Description

Components	Human TNF-α, premium grade: Purified recombinant human tumor necrosis factor α .
Size	10 μ g.
Biological activity	The ED ₅₀ is <0.025 ng/mL* corresponding to a specific activity of >4 \times 10 ⁷ I.U./mg.
Primary structure	Single, non-glycosylated polypeptide chain (157 amino acid residues).
Molecular mass	17.4 kDa.
Source	Produced in <i>E. coli</i> .
Product format	Lyophilized from a 0.2 μ m filtered solution.
Stabilizer	Trehalose and mannitol.
Purity	>97% as determined by chip-based gel electrophoresis.
Endotoxin level	Low endotoxin (<1 EU/ μ g cytokine) as determined by Limulus Amebocyte Lysate (LAL) assay.
Storage	Lyophilized Human TNF- α , premium grade should be stored at -20 °C. The expiration date is indicated on the vial label. Upon reconstitution aliquots should be stored at -20 °C. Avoid repeated freeze-thaw cycles.
Reconstitution	It is recommended to reconstitute lyophilized Human TNF- α with deionized sterile-filtered water up to a final concentration of 10 μ g/mL. ▲ Note: Addition of carrier protein, such as 0.1% bovine serum albumin (BSA) or human serum albumin (HSA) may have stabilizing effects. Further dilutions should be prepared with 1% BSA or HSA in phosphate-buffered saline (PBS).

* The ED₅₀ is determined by cytotoxicity assay using L929 cells in the presence of 1 μ g/mL actinomycin D according to Baarsch *et al.*¹. The cytotoxicity assay was calibrated with the first international reference standard for human TNF- α (NIBSC code 88/786) provided by the WHO/National Institute for Biological Standards and Control.

1.1 Background information

Tumor necrosis factor α (TNF- α), also called TNF ligand superfamily member 2 (TNFSF2), is a pro-inflammatory cytokine that is mainly produced by activated monocytes and macrophages in response to infection, injury, and tumor burden. TNF- α production has also been reported for a variety of other cell types involved in inflammatory responses, including T cells, NK cells, and neutrophils as well as a number of non-immune cells, such as keratinocytes and astrocytes. TNF- α has a broad spectrum of biological activities. In addition to its central role in inflammation, TNF- α is noted for its cytotoxic and tumoricidal abilities either by necrosis or apoptosis induction. Further functions include anti-viral activity, growth modulation, and induction of cellular differentiation.² Despite its various beneficial actions, TNF- α also plays a detrimental role in septic shock syndrome, tissue injury, inflammation, cachexia, diabetes, etc.³

TNF- α exists also as a membrane-associated 26 kDa precursor from which the mature soluble 17 kDa form is released by proteolytic cleavage mediated by TNF- α converting enzyme. TNF- α exerts many of its effects by binding to two distinct cell surface receptors, the 55 kDa TNF receptor 1 or the 75 kDa cell TNF receptor 2.⁴

1.2 Applications

- TNF- α can be used for a variety of applications, including induction of Mo-DC maturation, cytotoxicity and cell proliferation assays, assessment of apoptosis and viral protection, and investigation of TNF- α -induced signaling pathways.

Best concentration for a specific application should be determined by a dose-response experiment.

2. References

1. Baarsch, M. J. *et al.* (1991) Detection of tumor necrosis factor alpha from porcine alveolar macrophages using an L929 fibroblast bioassay. *J. Immunol. Methods* 140: 15–22.
2. Barbara, J. A. *et al.* (1996) Tumour necrosis factor-alpha (TNF-alpha): the good, the bad and potentially very effective. *Immunol. Cell. Biol.* 74: 434–443.
3. Yeung, M. C. *et al.* (1996) An essential role for the interferon-inducible, double-stranded RNA-activated protein kinase PKR in the tumor necrosis factor-induced apoptosis in U937 cells. *Proc. Natl. Acad. Sci. U S A.* 93: 12451–12455.
4. Black, R. A. *et al.* (1997) A metalloproteinase disintegrin that releases tumour necrosis factor-alpha from cells. *Nature* 385: 729–733

All protocols and data sheets are available at www.miltenyibiotec.com.

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