

Recombinant Rat Tumor Necrosis Factor-alpha (TNF-alpha)

Certificate of Analysis and Data Sheet

➤ Source: E.Coli	➤ Catalog No. CTK-393
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➤ **Background:**

TNF is secreted by macrophages, monocytes, neutrophils, T-cells, NK-cells following their stimulation by bacterial LPS. Cells expressing CD4 secrete TNF-alpha while CD8 cells secrete little or no TNF-alpha. The synthesis of TNF-alpha is induced by many different stimuli including interferons, IL2, GM-CSF.

➤ **Description :**

Recombinant rat TNF-alpha produced in E.Coli is a single, non-glycosylated, polypeptide chain containing 157 amino acids and having a molecular mass of 17339.44 Dalton.

Recombinant Rat TNF-alpha is purified by standard chromatographic techniques.

➤ **Physical Appearance:**

Sterile Filtered White lyophilized (freeze-dried) powder.

➤ **Formulation:**

The protein was lyophilized with no additives.

➤ **Solubility:**

It is recommended to reconstitute the lyophilized Rat TNF-alpha in sterile 18MΩ-cm H₂O not less than 100µg/ml, which can then be further diluted to other aqueous solutions.

➤ **Stability:**

Lyophilized Rat TNF-alpha although stable at room temperature for 3 weeks, should be stored desiccated below -18°C. Upon reconstitution Rat TNF-alpha should be stored at 4°C between 2-7 days and for future use below -18°C. For long-term storage it is recommended to add a carrier protein (0.1% HSA or BSA).

Please prevent freeze-thaw cycles.

➤ **Purity:**

Greater than 97.0% as determined by:

- (a) Analysis by RP-HPLC.
- (b) Anion-exchange FPLC.
- (c) Analysis by reducing and non-reducing SDS-PAGE Silver Stained gel.

➤ **Amino acid sequence:**

The sequence of the first five N-terminal amino acids was determined and was found to be Met-Leu-Arg-Ser-Ser.

➤ **Dimers and aggregates:**

Less than 1% as determined by silver-stained SDS-PAGE gel analysis.

➤ **Biological Activity:**

This Rat TNF-alpha is fully biologically active when compared to standard. The ED₅₀ as determined by the cytolysis of murine L929 cells in the presence of Actinomycin D is < 0.05ng/ml, corresponding to a Specific Activity of 5x10⁷ IU/mg.

➤ **Endotoxin:**

Less than 0.1 ng/μg (IEU/μg) of Rat TNF-alpha.

➤ **Protein content:**

Protein quantitation was carried out by two independent methods:

1. UV spectroscopy at 280 nm using the absorbency value of 1.25 as the extinction coefficient for a 0.1% (1mg/ml) solution. This value is calculated by the PC GENE computer analysis program of protein sequences (IntelliGenetics).
2. Analysis by RP-HPLC, using a calibrated solution of TNF-a as a Reference Standard.

➤ **Usage:**

This material is offered for research, laboratory or further evaluation purposes.