

Recombinant Human Tumor Necrosis Factor-alpha Mutant/Variant

Catalog No.	CRT018A	Quantity:	10 µg
	CRT018B		50 µg
	CRT018C		1 mg

Description: 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, IL-2, GM-CSF. The clinical use of the potent anti-tumor activity of TNF-alpha has been limited by the proinflammatory side effects including fever, dose-limiting hypotension, hepatotoxicity, intravascular thrombosis, and hemorrhage. Designing clinically applicable TNF-alpha mutants with low systemic toxicity has been an intense pharmacological interest. Human TNF- α , which binds to the mouse TNF-R55 but not to the mouse TNF-R75, exhibits retained anti-tumor activity and reduced systemic toxicity in mice compared with mouse TNF-alpha, which binds to both mouse TNF receptors. Based on these results, many TNF- α mutants that selectively bind to TNF-R 55 have been designed. These mutants displayed cytotoxic activities on tumor cell lines *in vitro*, and exhibited lower systemic toxicity *in vivo*. Recombinant Human TNF-alpha Variant/Mutant compared with the wildtype, has an amino acid sequence deletion from a.a. 1-7, and the following a.a. substitutes Arg 8, Lys 9, Arg 10 and Phe 157 which is proven to have more activity and with less inflammatory side effect *in vivo*.

Recombinant Human TNF-alpha Variant produced in *E. coli* is a single, non-glycosylated, polypeptide chain containing 151 amino acids and having a MW = 16.598 kDa. The Human TNF-alpha Variant is purified by standard chromatographic techniques.

Source: *E. coli*

Formulation: The protein was lyophilized after extensive dialysis against 0.5 x PBS, pH 7.0.

Purity: Greater than 95.0% as determined by analysis by RP-HPLC, anion-exchange FPLC and by analysis by reducing and non-reducing SDS-PAGE Silver Stained gel.



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- Endotoxin Level:** Less than 0.1 ng/μg (1 EU/μg) of Human TNF-alpha.
- Dimers & Aggregates:** Less than 1% as determined by silver-stained SDS-PAGE gel analysis.
- Biological Activity:** Recombinant Human TNF-alpha is Variant fully biologically active when compared to the wild type. The ED₅₀ as determined by the cytotoxicity of mouse L929 cells in the presence of Actinomycin D is < 0.05 ng/ml, corresponding to a Specific Activity of 1 x 10⁸ IU/mg.
- Amino Acid Sequence:** The sequence of the first five N-terminal amino acids is MRKRKPVAHV VANPQAEGQL QWLNRRANAL LANGVELRDN QLVVPSEGLY LIYSQVLFKG QGCPSTHVLL THTISRIAVS YQTKVNLLSA IKSPCQRETP EGAEAKPWYE PIYLGGVFQL EKGDRLSAEI NRPDYLDFAE SGQVYFGIIAF.
- Reconstitution:** **Centrifuge vial prior to opening.** It is recommended to reconstitute the lyophilized Recombinant Human TNF-alpha Variant in sterile distilled H₂O not less than 100 μg/ml, which can then be further diluted to other aqueous solutions.
- Storage & Stability:** Lyophilized Recombinant Human TNF-alpha Variant, although stable at room temperature for 3 weeks, should be stored desiccated below -18°C. Upon reconstitution Human TNF-alpha Variant 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). **Avoid repeated freeze-thaw cycles.**
- Protein Content:** Protein quantitation was carried out by two independent methods:
1. UV spectroscopy at 280 nm.
2. Analysis by RP-HPLC, using a calibrated solution of TNF-alfa as a Reference Standard.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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