

## Technical Data Sheet

## Recombinant Mouse TNF

## Product Information

Material Number:	554589
Size:	10 µg
Concentration:	200 µg/ml
Reactivity:	QC Testing: Mouse
Storage Buffer:	Frozen aqueous buffered solution containing BSA.

## Description

Tumor Necrosis Factor (TNF, aka TNF- $\alpha$ ) is a potent multifunctional cytokine which can exert regulatory and cytotoxic effects on a wide range of normal lymphoid and non-lymphoid cells and tumor cells. Mouse TNF is a differentially glycosylated protein containing 156 amino acid residues. Recombinant mouse TNF (Cat. No. 554589) is supplied as a frozen liquid comprised of 0.22 µm sterile-filtered aqueous buffered solution containing bovine serum albumin, with no preservatives. Recombinant mouse TNF is  $\geq$  95% pure as determined by SDS-PAGE, and an absorption assay based on the Beers-Lambert Law. The endotoxin level is  $\leq$  0.1 ng per µg of mouse TNF, as measured in a chromogenic LAL assay.

## Preparation and Storage

Store product at -80°C prior to use or for long term storage of stock solutions.

Rapidly thaw and quick-spin product prior to use.

Avoid multiple freeze-thaws of product.

This preparation contains no preservatives, thus it should be handled under aseptic conditions.

## Application Notes

## Application

ELISA Standard	Routinely Tested
Bioassay	Tested During Development
Blocking	Tested During Development

## Recommended Assay Procedure:

Upon initial thawing, recombinant mouse TNF (Cat. No. 554589) should be aliquoted into polypropylene microtubes and frozen at -80°C for future use. Alternatively, the product can be diluted in sterile neutral buffer containing not less than 0.5 - 10 mg/mL carrier protein, such as human or bovine serum albumin, aliquoted and stored at -80°C. For *in vitro* biological assay use, carrier protein concentrations of  $\geq$  1 mg/mL are recommended. For use as an ELISA standard, carrier protein concentrations of 5 - 10 mg/mL are recommended. Recombinant mouse TNF (Cat. No. 554589) should not be diluted to less than 50 µg/mL for long term storage. Failure to add carrier protein or store at indicated temperatures may result in a loss of activity. Carrier proteins may have an undesired influence on experimental results due to toxicity, high endotoxin levels or possible blocking activity.

**ELISA Standard:** Recombinant mouse TNF (Cat. No. 554589) can be useful as a quantitative standard for measuring mouse TNF protein levels using sandwich ELISA with purified G281-2626 antibody (Cat. No. 551225) as a capture antibody and biotinylated MP6-XT3 (Cat. No. 554415) as the detection antibody. To obtain linear standard curves, investigators may want to consider using doubling dilutions of recombinant mouse TNF from 2,000 - 15 pg/mL to be included in each ELISA plate. For measuring mouse TNF in serum or plasma, investigators are highly encouraged to use the BD OptEIA™ Mouse TNF ELISA Set (Cat. No. 555268 or 558534) or BD OptEIA™ Mouse TNF ELISA Kit (Cat. No. 560478).

**Bioassay:** Investigators are advised that the Bioassay application is not routinely tested for this material and are highly encouraged to both titrate this material and include appropriate controls in relevant experiments. An activity range of 0.2 - 5.0 x 10<sup>9</sup> units/mg, encompassing an ED50 = 2 - 50 pg/mL, has previously been reported using L929 indicator cells for proliferation, with a unit defined as the amount of material needed to stimulate a half-maximal response at cytokine saturation.

**Blocking:** Recombinant mouse TNF (Cat. No. 554589) can be useful as a blocking control for flow cytometric analysis when used with a fluochrome conjugated-MP6-XT22 antibody (such as PE conjugated MP6-XT22 ; Cat. No. 554419). Investigators are advised that the blocking application is not routinely tested for this material. Intracellular cytokine staining techniques and the use of blocking controls are described in detail by C. Prussin and D. Metcalfe.

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## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
554415	Biotin Rat Anti-Mouse TNF	0.5 mg	MP6-XT3
551225	Purified Rat Anti-Mouse/Rat TNF	1.0 mg	G281-2626
555268	Mouse TNF (Mono/Mono) ELISA Set	20 plates	(none)
558534	Mouse TNF ELISA Set II	20 plates	(none)
560478	Mouse TNF ELISA Kit	2 plates	(none)

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Please refer to [www.bdbiosciences.com/pharming/en/protocols](http://www.bdbiosciences.com/pharming/en/protocols) for technical protocols.

## References

Hogan MM, Vogel SN. Production of tumor necrosis factor by rIFN-gamma-primed C3H/HeJ (Lpsd) macrophages requires the presence of lipid A-associated proteins. *J Immunol.* 1988; 141(12):4196-4202. (Methodology: Bioassay)

Jaattela, M.. Biologic activities and mechanisms of action of tumor necrosis factor- $\alpha$ /cachectin. *Lab Invest.* 1991; 64:724-742. (Biology)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128. (Methodology)

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