Technical Data Sheet

# Purified Rat Anti-Mouse TNF

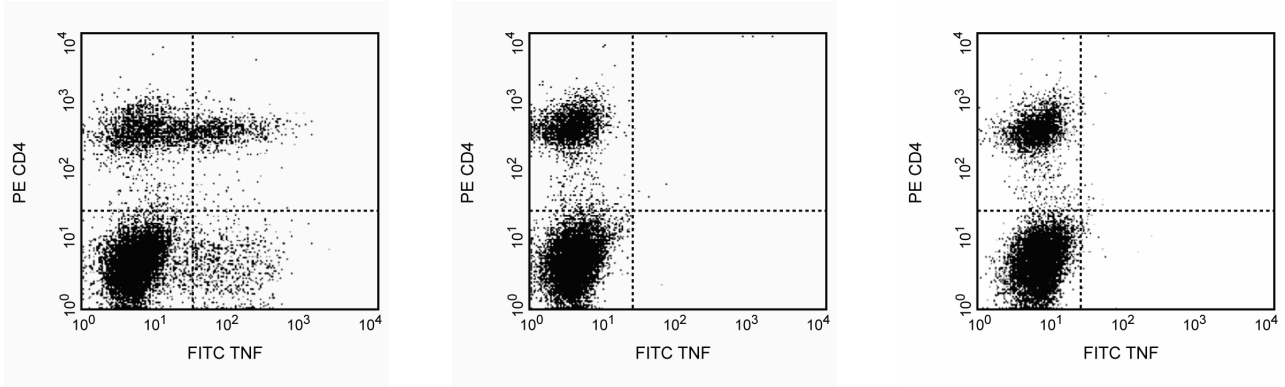
## Product Information

| | |
|---|---|
| **Material Number:** | **554416** |
| **Size:** | 0.1 mg |
| **Concentration:** | 0.5 mg/ml |
| **Clone:** | MP6-XT22 |
| **Immunogen:** | Recombinant mouse TNF |
| **Isotype:** | Rat IgG1 |
| **Reactivity:** | QC Testing: Mouse |
| **Storage Buffer:** | Aqueous buffered solution containing ≤0.09% sodium azide. |

## Description

The MP6-XT22 antibody reacts with mouse tumor necrosis factor (TNF, also known as TNF-α). The immunogen used to generate this hybridoma was recombinant mouse TNF.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.




***Expression of TNF by stimulated CD4+ and CD4- BALB/c spleen cells.*** *After 6 hour stimulation with hamster anti-mouse CD3 (2 µg/ml final concentration; Clone 145-2C11, Cat. No. 553057) and hamster anti-mouse CD28 (2 µg/ml final concentration; Clone 37.51, Cat. No. 553294) antibodies in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724), the splenocytes were stained with FcBlock™ (1 µg/1 million cells; Cat No. 553142), then 0.06 µg of PE-conjugated rat anti-mouse CD4 (PE-RM4-5, Cat. No. 553049). The cells were then fixed, permeabilized, and subsequently stained with 0.06 µg of FITC-conjugated rat anti-mouse TNF antibody (FITC-MP6-XT22, Cat. No. 554418) by using Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding of the FITC-MP6-XT22 antibody was blocked by preincubation of the antibody conjugate with recombinant mouse TNF (0.25 µg, Cat. No. 554589; middle panel), and by preincubation of the fixed/permeabilized cells with unlabeled MP6-XT22 antibody (2 µg, Cat.No. 554416; right panel) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (middle panel) and antibody blocking (right panel) specificity controls.*

## Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

## Application Notes

**Application**

| | |
|---|---|
| Intracellular block/flow cytometry | Routinely Tested |
| ELISA Capture | Tested During Development |
| Western blot | Reported |

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**Recommended Assay Procedure:**

**Blocking Control for Intracellular Staining:** The purified MP6-XT22 antibody (Cat. No. 554416) can be used as a blocking control to demonstrate specificity of TNF protein staining by directly conjugated -MP6-XT22 antibody. To perform this control, the fixed/permeabilized cells (~ 1 million) can be incubated with 1 -10 µg of unlabeled MP6-XT22 antibody (Cat. No. 554416) for 20 minutes at 4°C, prior to staining with directly conjugated antibody (e.g., 0.1 -0.5 µg mAb/1 million cells). The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

**ELISA Capture:** The purified MP6-XT22 antibody (Cat. No. 554416) is useful as a capture antibody for a sandwich ELISA for measuring mouse TNF protein levels. Purified MP6-XT22 antibody can be paired with the biotinylated polyclonal rabbit anti-mouse/rat TNF antibody (Cat. No 557432) as the detecting antibody, with recombinant mouse TNF (Cat. No. 554589) as the standard. This pair measures total TNF, free TNF as well as TNF bound by soluble receptors.

***Note :*** This ELISA pair is recommended primarily for measuring cytokine from experimental cell culture systems. These ELISA reagents are not recommended for assaying serum or plasma samples. For measuring Mouse TNF in serum or plasma our Mouse TNF BD OptEIA™ Set (Cat. No. 558534) or BD OptEIA Kit (Cat. No. 559732) are specially formulated and recommended.

**WB:** The MP6-XT22 antibody has been reported to be useful for Western blotting. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

## Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|---|---|---|---|
| 554715 | BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop) | 250 tests | (none) |
| 554589 | TNF Recombinant Mouse | 10 µg | (none) |
| 554652 | MiCK-1 Cytokine Positive Control Cells | 5x10^6 cells | (none) |
| 558534 | Mouse TNF ELISA Set Reagents for 20 plates | 20 plates | (none) |
| 554419 | PE Rat Anti-Mouse TNF | 0.1 mg | MP6-XT22 |
| 554418 | FITC Rat Anti-Mouse TNF | 0.1 mg | MP6-XT22 |
| 557432 | Biotin Rabbit Anti-Mouse/Rat TNF | 0.5 mg | C1150-14 |

## Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

## References


Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. In: Coligan J, Kruisbeek A, Margulies D, Shevach E, Strober W, ed. *Current Protocols in Immunology.* New York: John Wiley and Sons; 1995:6.20-6.21.(Clone-specific)

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24.(Clone-specific)

Litton MJ, Sander B, Murphy E, O'Garra A, Abrams JS. Early expression of cytokines in lymph nodes after treatment in vivo with Staphylococcus enterotoxin B. *J Immunol Methods.* 1994; 175(1):47-58.(Clone-specific)

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)