Technical Data Sheet

FITC Rat Anti-Mouse TNF

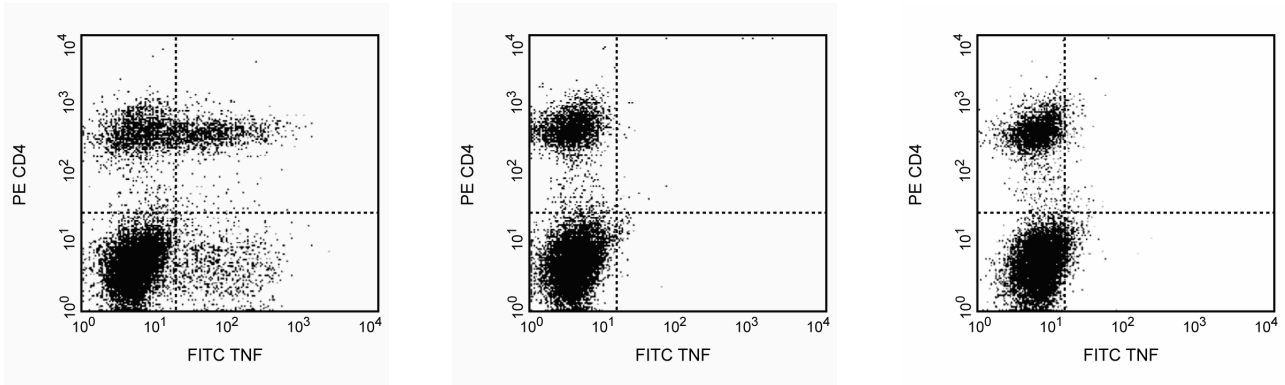
Product Information

Material Number:	**561064**
Size:	25 µg
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 h stimulation with hamster anti-mouse CD3 (Clone 145-2C11, 2 µg/ml, Cat. No. 553057) and hamster anti-mouse CD28 (Clone 37.51, 2 µg/ml, Cat. No. 553294) antibodies in the presence of BD GolgiStop™ (Cat. No. 554724; aka monensin, 3 µM) the splenocytes were stained with mouse BD 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, 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 (0.06 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.
The antibody was conjugated with FITC under optimum conditions, and unreacted FITC was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometry: The FITC-conjugated MP6-XT22 antibody can be used for multicolor immunofluorescent staining and flow cytometric analyses to identify and enumerate TNF producing cells within mixed cell populations (see figure). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). 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.

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A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated MP6-XT22 antibody with ligand (e.g., recombinant mouse TNF; Cat. No 554589) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabeled MP6-XT22 antibody (Cat. No 554416) prior to staining. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse cells is FITC-R3-34 (Cat. No 554684); use at comparable concentrations to antibody of interest.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554416	Purified Rat Anti-Mouse TNF	0.1 mg	MP6-XT22
554684	FITC Rat IgG1, κ Isotype Control	0.1 mg	R3-34
553057	Purified NA/LE Hamster Anti-Mouse CD3e	0.5 mg	145-2C11
553294	Purified NA/LE Hamster Anti-Mouse CD28	0.5 mg	37.51
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)

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.
4. Use of these products to measure activation antigens expressed on mononuclear cell subsets for the purpose of monitoring immunoregulatory status can fall under one or more claims of the following patents: US Patent Nos. 5,445,939, 5,656,446, 5,843,689; European Patent No. 319,543; Canadian Patent No. 1,296,622; Australian Patent No. 615,880; and Japanese Patent No. 2,769,156.

References

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)

Hunter CA, Litton MJ, Remington JS, Abrams JS. Immunocytochemical detection of cytokines in the lymph nodes and brains of mice resistant or susceptible to toxoplasmic encephalitis. *J Infect Dis.* 1994; 170(4):939-945. (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)