Technical Data Sheet

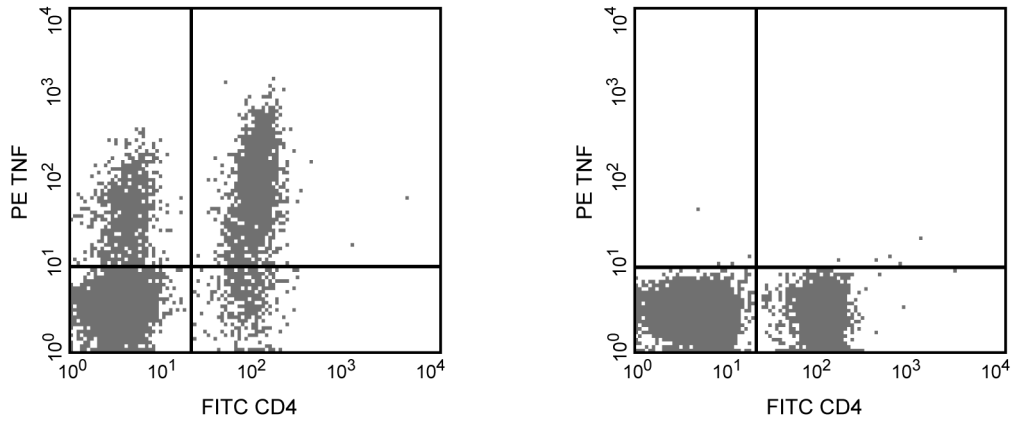
PE Rat Anti-Mouse TNF

Product Information

Material Number:	**554419**
Size:	0.1 mg
Concentration:	0.2 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.



Expression of TNF by MiCK-1 cells. *MiCK-1 cells (Cat. No. 554652) were stained with 0.06 ug of FITC-conjugated rat anti-mouse CD4 (FITC-RM4-5, Cat. No. 553047), fixed, permeabilized, and subsequently stained with 0.06 ug of PE-conjugated rat anti-mouse TNF antibody (PE-MP6-XT22, Cat. No. 554419) by using the Pharmingen staining protocol (left panel). To demonstrate specificity of staining, the binding of PE-MP6-XT22 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant mouse TNF (0.25 µg, Cat. No. 554589; right panel), and by preincubation of the fixed/permeabilized cells with an excess of the unlabelled MP6-XT22 mAb (2 µg, Cat. No. 554416; data not shown). The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking (right panel) and unlabelled antibody blocking specificity controls.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were 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:

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The PE-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.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated MP6-XT22 antibody with a molar excess of ligand (e.g., recombinant mouse TNF; Cat No. 554589) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabelled

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MP6-XT22 antibody (Cat. No. 554416) prior to staining. The staining technique and 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 and human cells is PE-R3-34 (Cat. No. 554685); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/ 1 million cells).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554685	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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 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)