Technical Data Sheet

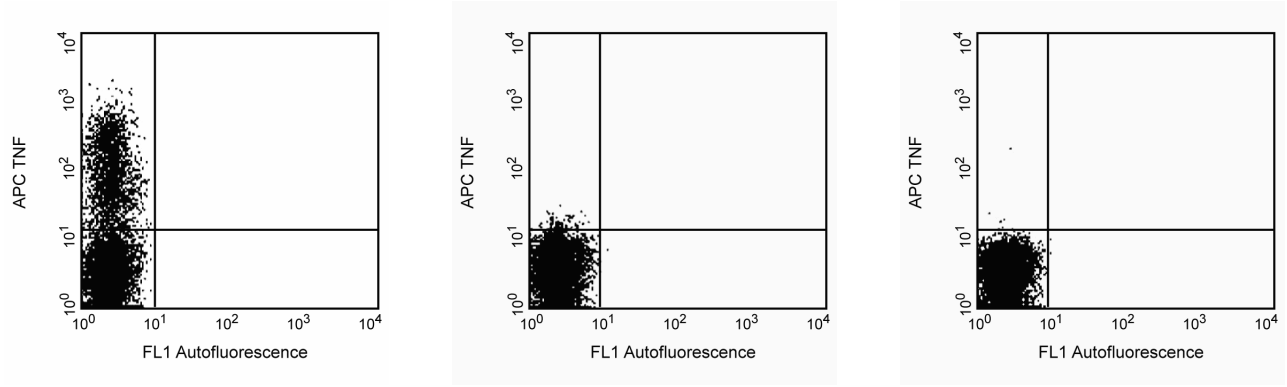
APC Rat Anti-Mouse TNF

Product Information

Material Number:	**561062**
Size:	25 µg
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 stimulated BALB/c spleen cells. *After a 4 hour stimulation with PMA (5.0 ng/ml final concentration; Sigma) and ionomycin (500 ng/ml final concentration; Sigma) in the presence of GolgiPlug™ (1.0 µg/ml final concentration; Cat. No. 555029) the splenocytes were stained with FcBlock™ (1.0 µg/1 million cells; Cat No. 553142). The cells were then fixed, permeabilized, and subsequently stained with 0.03 µg of APC-conjugated rat anti-mouse TNF antibody (APC-MP6-XT22, Cat. No. 554420) by using Pharmingen's staining protocol (see Figure, left panel). To demonstrate specificity of staining, the binding of the APC-MP6-XT22 antibody was blocked by preincubation of the antibody conjugate with recombinant mouse TNF (0.25 µg, Cat. No. 554589; see middle panel), and by preincubation of the fixed/permeabilized cells with unlabeled MP6-XT22 antibody (5.0 µg, Cat. No. 554416; see 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. This APC-conjugated reagent can be used in any flow cytometer equipped with a a dye, HeNE or red diode laser. These include the dual laser FACStarPLUS™, FACS Vantage™ or FACSCalibur™.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometry: The APC-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 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 APC-R3-34 (Cat. No. 554686). Isotype controls should be used at a comparable concentration to the antibody of interest.

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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.

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
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
554589	Recombinant Mouse TNF	10 µg	(none)
554416	Purified Rat Anti-Mouse TNF	0.1 mg	MP6-XT22
554686	APC Rat IgG1, κ Isotype Control	0.1 mg	R3-34

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.

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. (Biology)

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. (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: Flow cytometry)