Technical Data Sheet

Alexa Fluor® 647 Rat Anti-Mouse TNF

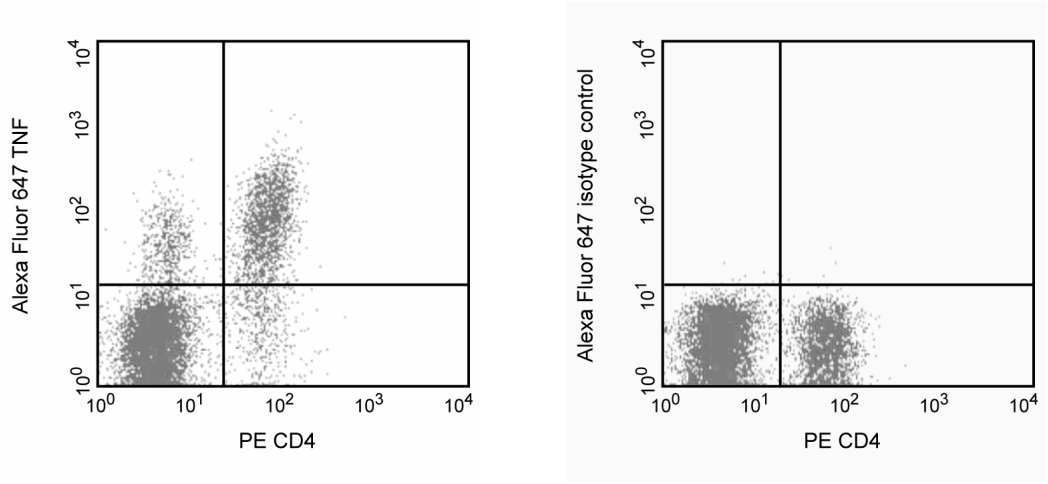
Product Information

Material Number:	**557730**
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.

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. *Splenocytes from BALB/C mice were stimulated for 4 hrs with PMA (5 ng/ml, Sigma) and Ionomycin (500 ng, Sigma) in the presence of BD GolgiPlug™ (Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with PE-conjugated rat anti-mouse CD4 (PE-RM4-5, Cat. No. 553048) and either rat anti-mouse TNF antibody (Alexa 647-MP6-XT22, Cat. No. 557730), (left panel) or immunoglobulin isotype control (Alexa 647-R3-34, Cat. No. 557731), (right panel) by using the BD Pharmingen staining protocol. To demonstrate specificity of staining the binding of Alexa 647-MP6-XT22 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant mouse TNF (0.25 µg, Cat. No. 554589, data not shown) and by preincubation of the fixed/permeabilized cells with an excess of unlabelled MP6-XT22 antibody (5 µg, Cat. No. 554416, data not shown) prior to stainining. Dot plots were derived from gated events with the forward and side light scatter characteristics of lymphocytes. The quadarant markers for the bivariate dot plots were set based on the autofluorescence and isotype controls.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 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:

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The FITC-, PE-, APC-, and PE-Cy7-, Alexa Fluor® 488- and Alexa Fluor® 647-conjugated MP6-XT22 antibody (Cat No. 554418, 554419,

BD Biosciences

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554420, 557644, 557719 and 557730) are available for multicolor immunofluorescent staining and flow cytometric analysis 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 the protocols section or chapter on intracellular flow cytometry in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

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 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 Alexa Fluor® 647-R3-34 (Cat. No. 557731). Use isotype controls at comparable concentrations to antibody of interest.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557731	Alexa Fluor® 647 Rat IgG1, κ Isotype Control	0.1 mg	R3-34
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
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	5x10^6 cells	(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.
5. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
6. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
7. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

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)