Technical Data Sheet

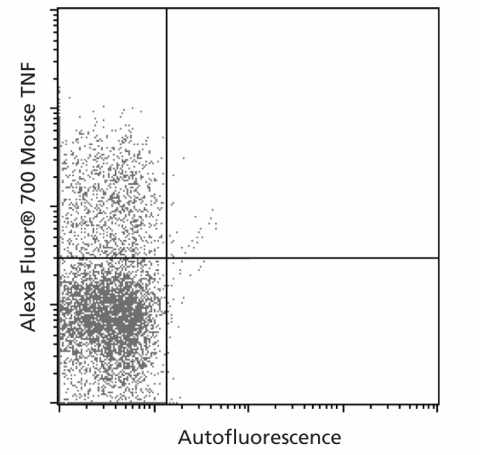
Alexa Fluor® 700 Rat anti-Mouse TNF

Product Information

Material Number:	**558000**
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 protein stabilizer and ≤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. *Splenocytes from BALB/c mice were stimulated for 4 hrs with PMA (5 ng/ml, Sigma, Cat. No. P-8139) and Ionomycin (500 ng, Sigma, Cat. No. I0634) in the presence of Brefeldin A (GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with Alexa Fluor® 700 Rat anti-Mouse TNF or Alexa Fluor® 700 Rat IgG1 κ Isotype Control (data not shown). Dot plots were derived from gated events with the forward and side light scatter characteristics of lymphocytes. The quadrant markers for the bivariate dot plot was 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® 700 under optimum conditions, and unreacted Alexa Fluor® 700 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 Alexa Fluor® 700-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 Alexa Fluor® 700 Rat IgG1 κ Isotype Control (Cat. No 558001); use at comparable concentrations to antibody of interest.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558001	Alexa Fluor® 700 Rat IgG1 κ Isotype Control	0.1 mg	R3-34

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

554589	Recombinant Mouse TNF	10 µg	(none)
554416	Purified Rat Anti-Mouse TNF	0.1 mg	MP6-XT22
554652	MiCK-1 Mouse Cytokine Positive Control Cells	5x10^6 cells	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	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. Alexa Fluor® 700 has an adsorption maximum of ~700nm and a peak fluorescence emission of ~720nm. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
5. 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.
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. 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.
8. 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)