Technical Data Sheet

Alexa Fluor® 488 Mouse Anti-Human TNF

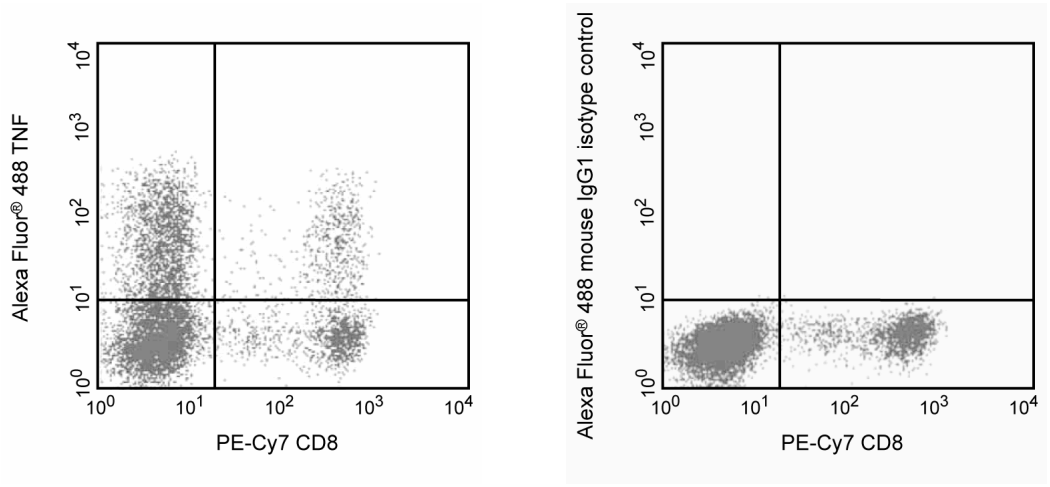
Product Information

Material Number:	**557722**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	MAb11
Immunogen:	Recombinant Human TNF
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The MAb11 antibody reacts with human tumor necrosis factor (TNF, also known as TNF-α) protein. TNF is an efficient paracrine and endocrine mediator of inflammatory and immune functions. It regulates the growth and differentiation of a variety of cell types. TNF is cytotoxic for transformed cells when in conjunction with IFN-γ. It is secreted by activated monocytes/macrophages and other cells such as B cells, T cells and fibroblasts. The immunogen used to generate the MAb11 hybridoma was recombinant human TNF. The use of the MAb11 antibody has been reported to cross-react with TNF of rhesus monkey.

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 human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells were stimulated for 4 hrs with PMA (5 ng/ml, Sigma, Cat. No. P-8139) and Ionomycin (500 ng, Sigma, P-8139) in the presence of Brefeldin A (GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with PE-Cy7-conjugated mouse anti-human CD8 (PE-Cy7-RPA-T8, Cat. No. 557746) and either mouse anti-human TNF antibody (Alexa 488-MAb11, Cat. No. 557722), (left panel) or immunoglobulin isotype control (Alexa 488-MOPC-21, Cat. No. 557721), (right panel) by using Pharmingen's staining protocol. To demonstrate specificity of staining the binding of Alexa 488-MAb11 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant human TNF (0.25 µg, Cat. No. 554618, data not shown) and by preincubation of the fixed/permeabilized cells with an excess of unlabelled MAb11 antibody (5 µg, Cat. No. 554510, 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® 488 under optimum conditions, and unreacted Alexa Fluor® 488 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:

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/**

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Immunofluorescent Staining and Flow Cytometric Analysis: The MAb11 antibody is useful for intracellular immunofluorescent staining and flow cytometric analysis to identify and enumerate TNF producing cells within mixed cell populations. The Alexa Fluor® 488-conjugated MAb11 antibody (Cat. No. 557722) is especially suitable for these studies (see image). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be used at 5 µl/test. The staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. 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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
557721	Alexa Fluor® 488 Mouse IgG1 κ Isotype Control	100 tests	MOPC-21
555061	HiCK-1 Human Cytokine Positive Control Cells	5x10^6 cells	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
5. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
6. 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.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. 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.
9. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.

References

Danis VA, Franic GM, Rathjen DA, Brooks PM. Effects of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-2, interferon-gamma (IFN-gamma), tumour necrosis factor-alpha (TNF-alpha) and IL-6 on the production of immunoreactive IL-1 and TNF-alpha by human monocytes. *Clin Exp Immunol.* 1991; 85(1):143-150.(Clone-specific: ELISA)

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)

Rathjen DA, Cowan K, Furphy LJ, Aston R. Antigenic structure of human tumour necrosis factor: recognition of distinct regions of TNF alpha by different tumour cell receptors. *Mol Immunol.* 1991; 28(1-2):79-86.(Clone-specific: ELISA)