Technical Data Sheet

FITC Mouse Anti-Human TNF

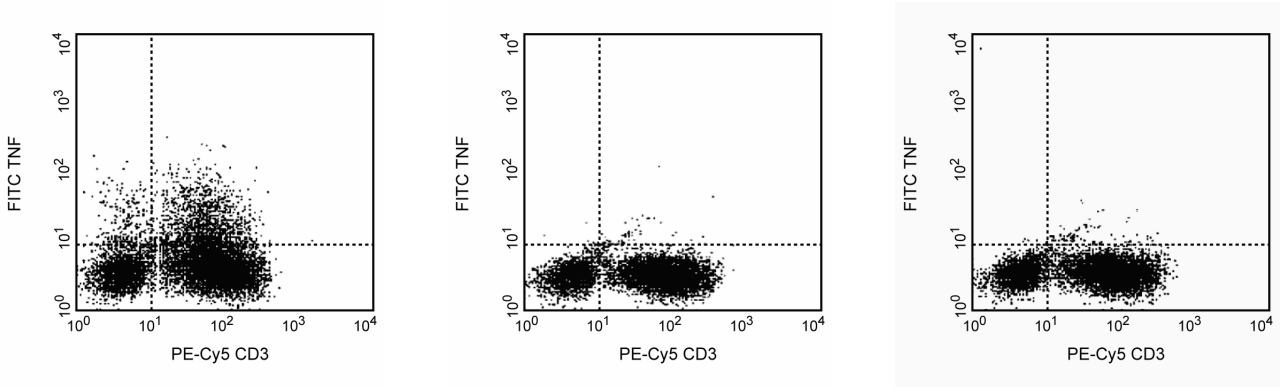
Product Information

Material Number:	**554512**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	MAb11
Immunogen:	Recombinant Human TNF
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The MAb11 antibody reacts with human tumor necrosis factor (TNF, formerly 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 mononuclear cells (PBMC). *Human PBMC were stimulated for 6 hours with PMA (Sigma, Cat. No. P-8139) and calcium ionophore A23187 (Sigma, Cat. No. C-9275) in the presence of GolgiStop™ (aka 2 µM monensin, Cat No. 554724). The PBMC were stained with PE-Cy5-anti-CD3 (PE-Cy5 -UCHT1, Cat. No. 555334), fixed, permeabilized, and subsequently stained with 0.25 µg of FITC mouse anti-human TNF (FITC-MAb11, Cat. No. 554512) by using BD Pharmingen staining protocol (left panel). To demonstrate specificity of staining, the binding of FITC-MAb11 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant human TNF (0.5 µg; Cat. No. 554618; middle panel), and by preincubation of the fixed/permeabilized cells with an excess of the unlabelled MAb11 antibody (10 µg, Cat. No. 554618, right panel) prior to staining with the FITC-MAb11 antibody. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking 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 FITC under optimum conditions, and unreacted FITC was removed.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

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Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

The FITC-conjugated MAb11 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 the protocols section or chapter on intracellular staining 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 MAb11 antibody with a molar excess of ligand (e.g., recombinant human TNF; Cat No. 554618) prior to staining, or 2) pre-block the fixed/ permeabilized cells with unlabelled MAb11 antibody (Cat. No. 554510) prior to staining. The staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is FITC-MOPC-21 (Cat. No. 554679); use at comparable concentrations to antibody of interest.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555061	HiCK-1 Cytokine Positive Control Cells	5x10^6 cells	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554679	FITC Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21

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. 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

Andersson J, Abrams J, Bjork L, et al. Concomitant in vivo production of 19 different cytokines in human tonsils. *Immunology.* 1994; 83(1):16-24.(Biology)

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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)

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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)

Raqib R, Lindberg AA, Wretlind B, Bardhan PK, Andersson U, Andersson J. Persistence of local cytokine production in shigellosis in acute and convalescent stages. *Infect Immun.* 1995; 63(1):289-296.(Biology)

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)