Technical Data Sheet

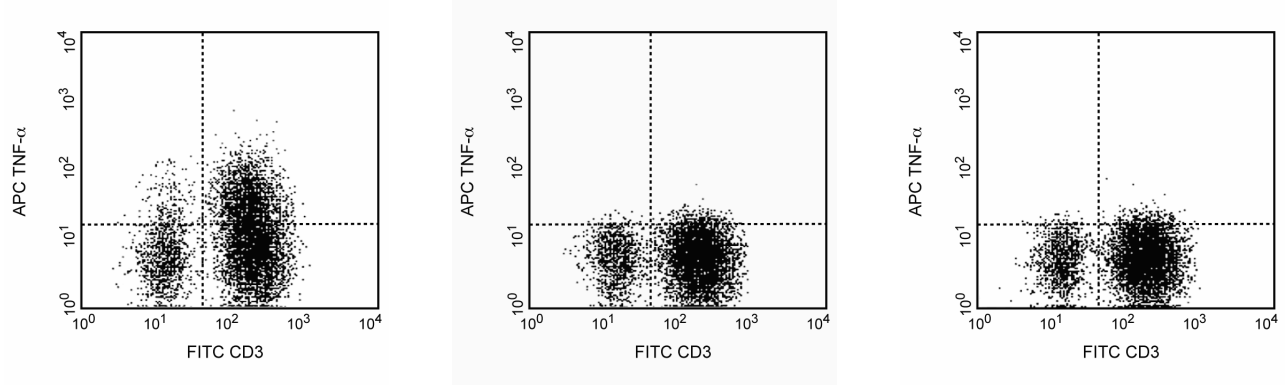
APC Mouse Anti-Human TNF

Product Information

Material Number:	**562084**
Alternate Name:	Tumor necrosis factor alpha; TNF-a; TNF-α; TNFSF2; Cachectin
Size:	25 µg
Concentration:	0.2 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 monoclonal antibody specifically binds to human tumor necrosis factor (TNF, also known as TNF-α) protein. TNF is an efficient juxtacrine, 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 MAb11 antibody has been reported to crossreact with Rhesus Macaque TNF.



Expression of TNF by stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated for 6 hours with PMA (Sigma, Cat. #P-8139) and calcium ionophore A23187 (Sigma, Cat. #C-9275) in the presence of BD GolgiStop™ (Cat. No. 554724; aka monensin 2 µM). The PBMC were stained with FITC-anti-CD3 (FITC-UCHT1, Cat. No. 555332), fixed, permeabilized, and subsequently stained with 0.25 µg of APC Mouse anti-Human TNF antibody by using the BD Biosciences staining protocol (left panel). To demonstrate specificity of staining, the binding of APC-MAb11 was blocked by the preincubation of the conjugated antibody with recombinant human TNF (0.5 µg; Cat. No. 554618; see middle panel), and by preincubation of the fixed/permeabilized cells with the unlabeled MAb11 antibody (10 µg, Cat. No. 554510; right panel) prior to staining with the APC-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 unlabeled antibody-blocking specificity controls.*

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 MAb11 antibody can be used for flow cytometric analysis to identify and enumerate TNF-producing cells within mixed cell populations (see image). 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.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

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

Conditions: The information disclosed herein is not to be construed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be held responsible for patent infringement or other violations that may occur with the use of our products. Purchase does not include or carry any right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton Dickinson and Company is strictly prohibited.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.

BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2011 BD

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated MAb11 antibody with a ligand (e.g., recombinant human TNF; Cat No. 554618) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabeled MAb11 antibody (Cat. No. 554510) prior to staining. The staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554618	Recombinant Human TNF	10 µg	(none)
554681	APC Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(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. This APC-conjugated reagent can be used in any flow cytometer equipped with a dye, HeNe, or red diode laser.
6. An isotype control should be used at the same concentration as the antibody of interest.

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)