Technical Data Sheet

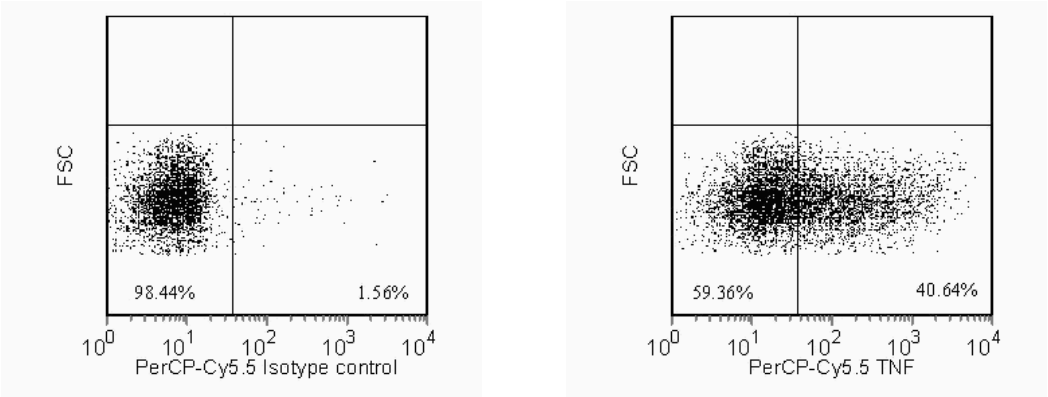
PerCP-Cy™5.5 Mouse Anti-Human TNF

Product Information

Material Number:	**560679**
Alternate Name:	Tumor necrosis factor alpha; TNF-a; TNF-α; TNFSF2; Cachectin
Size:	50 Tests
Vol. per Test:	5 µl
Clone:	MAb11
Immunogen:	Recombinant Human TNF
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon
Storage Buffer:	Aqueous buffered solution containing BSA and ≤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.



Flow cytometric analysis for TNF in stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated for 6 hours with 50 ng/mL PMA (Sigma-Aldrich Cat. No. P-8139) and 500 ng/mL calcium ionophore A23187 (Sigma-Aldrich Cat. No. C-9275) in the presence of BD GolgiStop™ (Cat. No. 554724). Cells were then fixed and permeabilized using BD Cytofix/Cytoperm™ (Cat. No. 554714) followed by staining with either a PerCP-Cy™5.5 Mouse IgG1, κ isotype control (Cat. No. 550795; left panel) or with the PerCP-Cy™5.5 Mouse Anti-Human TNF antibody (Cat. No. 560679; right panel). Dot plots were derived from gated events based on light scattering characteristics for lymphocytes. Flow cytometry was performed on a BD™ LSR II flow cytometry system.*

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 with PerCP-Cy5.5 under optimum conditions, and unconjugated antibody and free PerCP-Cy5.5 were removed. Storage of PerCP-Cy5.5 conjugates in unoptimized diluent is not recommended and may result in loss of signal intensity.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow cytometry: The MAb11 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate TNF producing cells within mixed cell populations. A useful control investigators may consider using for demonstrating specificity of staining, is to pre-block with one of the following reagents: (1) recombinant human TNF (Cat. No. 554618) or (2) unlabeled MAb11 antibody (Cat. No. 554510), prior to staining.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
550795	PerCP-Cy™5.5 Mouse IgG1 κ Isotype Control	0.1 mg	MOPC-21
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 Tests	(none)
555061	HiCK-1 Human Cytokine Positive Control Cells	1 mL	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 Tests	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 mL	(none)
554618	Recombinant Human TNF	10 µg	(none)
554510	Purified Mouse Anti-Human TNF	0.1 mg	MAb11
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(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. An isotype control should be used at the same concentration as the antibody of interest.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. PerCP-Cy5.5 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the tandem fluorochrome, extra care must be taken when using dual-laser cytometers, which may directly excite both PerCP and Cy5.5™. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
7. PerCP-Cy5.5–labelled antibodies can be used with FITC- and R-PE–labelled reagents in single-laser flow cytometers with no significant spectral overlap of PerCP-Cy5.5, FITC, and R-PE fluorescence.
8. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
10. Cy is a trademark of GE Healthcare.
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Black RA, Rauch CT, Kozlosky CJ, et al. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature.* 1997; 385(6618):729-733. (Biology)

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. (Biology)

Hogan MM, Vogel SN. Production of tumor necrosis factor by rIFN-gamma-primed C3H/HeJ (Lpsd) macrophages requires the presence of lipid A-associated proteins. *J Immunol.* 1988; 141(12):4196-4202. (Biology)

Jaattela, M. . Biologic activities and mechanisms of action of tumor necrosis factor-α/cachectin. *Lab Invest.* 1991; 64:724-742. (Biology)

Jason J, Larned J. Single-cell cytokine profiles in normal humans: comparison of flow cytometric reagents and stimulation protocols. *J Immunol Methods.* 1997; 207(1):13-22. (Biology)

Kriegler M, Perez C, DeFay K, Albert I, Lu SD. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. *Cell.* 1988; 53(1):45-53. (Biology)

Petyovka N, Lyach L, Voitenok NN. Homologous ELISA for detection of oligomeric human TNF: properties of the assay. *J Immunol Methods.* 1995; 186(2):161-170. (Biology)

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. (Biology)

Sander B, Andersson J, Andersson U. Assessment of cytokines by immunofluorescence and the paraformaldehyde-saponin procedure. *Immunol Rev.* 1991; 119:65-93. (Biology)

Smith RA, Baglioni C. The active form of tumor necrosis factor is a trimer. *J Biol Chem.* 1987; 262(15):6951-6954. (Biology)

Sopper S, Stahl-Hennig C, Demuth M, Johnston IC, Dorries R, ter Meulen V. Lymphocyte subsets and expression of differentiation markers in blood and lymphoid organs of rhesus monkeys. *Cytometry.* 1997; 29(4):351-362. (Biology)

Verdier F, Aujoulat M, Condevaux F, Descotes J. Determination of lymphocyte subsets and cytokine levels in cynomolgus monkeys. *Toxicology.* 1995; 105(1):81-90. (Biology)

Wang AM, Creasey AA, Ladner MB, et al. Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science.* 1985; 228(4696):149-154. (Biology)