Technical Data Sheet

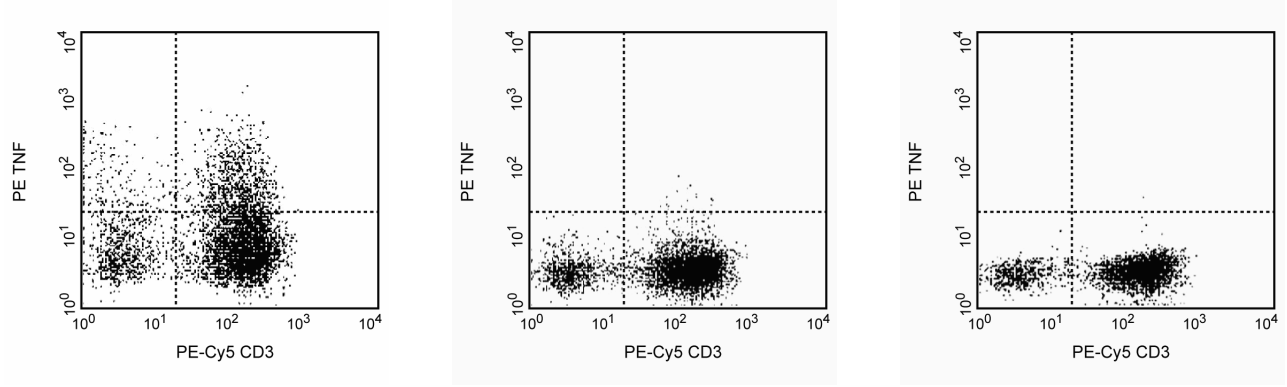
PE Mouse Anti-Human TNF

Product Information

Material Number:	**554513**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	MAb11
Immunogen:	Recombinant Human TNF
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Workshop:	NA
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 protein by stimulated human peripheral blood mononuclear cells (PBMC). *Human PBMC were stimulated for 6 hours with PMA (50 ng/ml final concentration; Sigma, Cat. #P-8139), calcium ionophore A23187 (0.5 µg/ml final concentration; Sigma, Cat. #C-9275) and PHA (Gibco BRL, Cat. #10576-015) in the presence of GolgiStop™ (2 µM final concentration; Cat. No. 554724). The PBMC were stained with 0.25 µg PE-Cy5-anti-CD3 (PE-Cy5-UCHT1, Cat. No. 555334) and subsequently stained with 0.5 µg of PE-conjugated mouse anti-human TNF antibody (PE-MAb11, Cat. No. 554513) by using Pharmingen's staining protocol (left panel). To demonstrate specificity of staining, the binding of PE-MAb11 was blocked by preincubation of the conjugate with excess (1 µg) recombinant human TNF (Cat. No. 554618; middle panel) and by preincubation of the fixed/permeabilized cells with an excess of unlabeled MAb11 antibody (Cat. No. 554510; right panel) prior to staining with the PE-Mab11 antibody. The quadrant markers for the bivariate dot plot was set based on the negative staining controls using isotype-matched Ig controls (PE-Cy7-mouse IgG1, Cat. No. 557646 and PE-mouse IgG1, Cat. No. 554680).*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were 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 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 (see image). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be pretitrated (≤ 1 µg mAb/million cells). 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.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554680	PE 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	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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.

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)