Technical Data Sheet

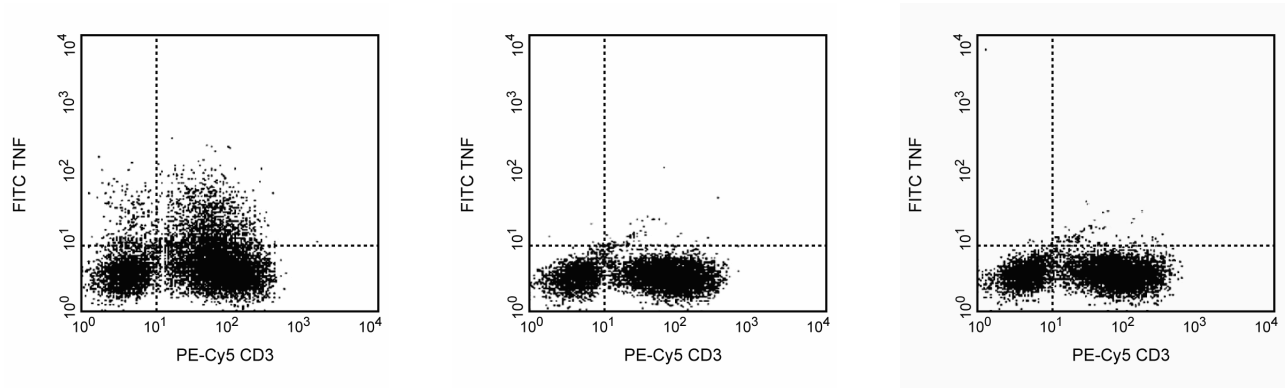
FITC Mouse Anti-Human TNF

Product Information

Material Number:	**562082**
Alternate Name:	Tumor necrosis factor alpha; TNF-a; TNF-α; TNFSF2; Cachectin
Size:	25 µg
Concentration:	0.5 mg/ml
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 ≤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. 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-Cy™5 Mouse Anti-Human CD3 (Cat. No. 555334), fixed, permeabilized, and subsequently stained with 0.25 µg of FITC Mouse Anti-Human TNF (Cat. No. 562082/554512; 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 Purified Mouse Anti-Human TNF antibody (10 µg, Cat. No. 554510; 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 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 with FITC under optimum conditions, and unreacted FITC was removed.

Application Notes

Application

Intracellular staining (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 under "Cytokines (Intracellular Staining)" or "Intracellular Flow" at our website, http://www.bdbiosciences.com/us/s/resources.

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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 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. 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
554618	Recombinant Human TNF	10 µg	(none)
554679	FITC 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)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
554512	FITC Mouse Anti-Human TNF	0.1 mg	MAb11
555334	PE-Cy™5 Mouse Anti-Human CD3	100 Tests	UCHT1
554510	Purified Mouse Anti-Human TNF	0.1 mg	MAb11

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
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.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.
6. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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