Technical Data Sheet

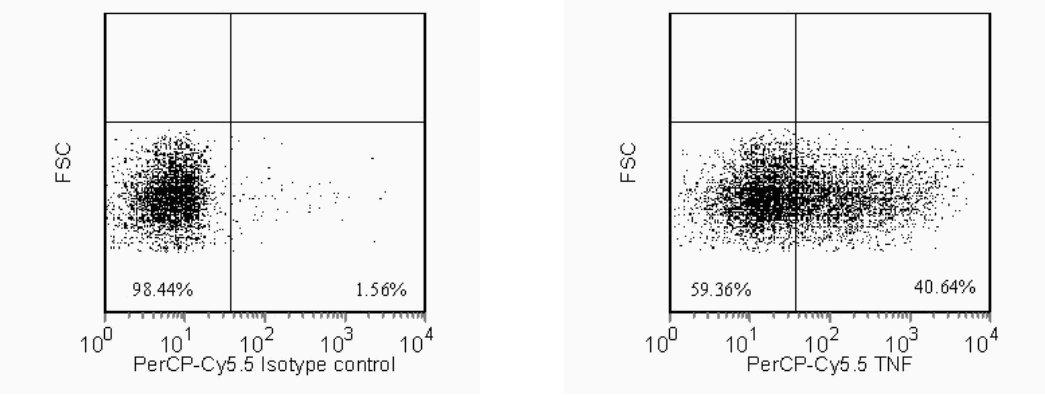
PerCP-Cy™5.5 Mouse Anti-Human TNF

Product Information

Material Number:	**560679**
Size:	50 tests
Vol. per Test:	5 µl
Clone:	MAb11
Immunogen:	Recombinant Human TNF
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
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 (left panel) or with the PerCP-Cy™5.5 Mouse Anti-Human TNF antibody (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	1.0 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

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. 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.
4. Cy is a trademark of Amersham Biosciences Limited. This conjugated product is sold under license to the following patents: US Patent Nos. 5,486,616; 5,569,587; 5,569,766; 5,627,027.
5. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University and made and sold under license from Amersham Biosciences Corp. This product is licensed for sale only for research. It is not licensed for any other use. If you require a commercial license to use this product and do not have one return this material, unopened to BD Biosciences, 10975 Torreyana Rd, San Diego, CA 92121 and any money paid for the material will be refunded.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. 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.
10. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
11. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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