

Technical Data Sheet

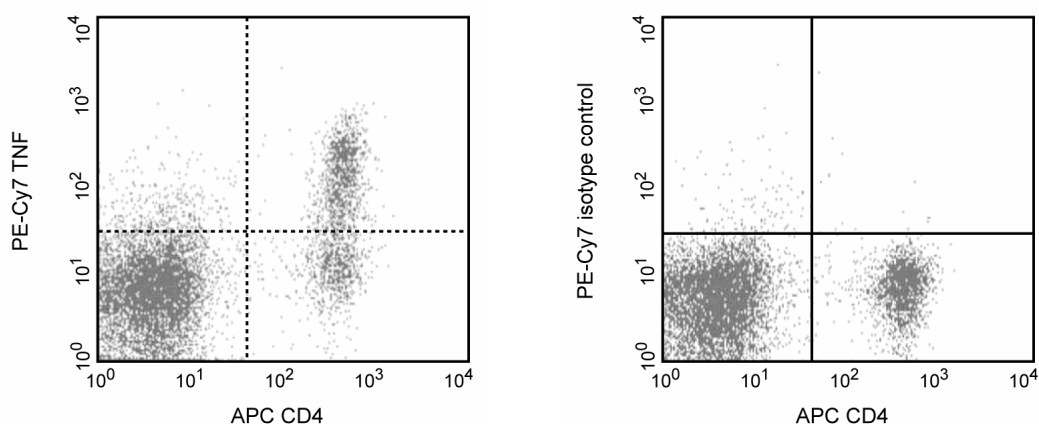
PE-Cy7™7 Rat Anti-Mouse TNF

Product Information

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| Material Number: | 557644 |
| Size: | 0.1 mg |
| Concentration: | 0.2 mg/ml |
| Clone: | MP6-XT22 |
| Immunogen: | Recombinant mouse TNF |
| Isotype: | Rat IgG1 |
| Reactivity: | QC Testing: Mouse |
| Storage Buffer: | Aqueous buffered solution containing ≤0.09% sodium azide. |

Description

The MP6-XT22 antibody reacts with mouse tumor necrosis factor (TNF, also known as TNF- α). The immunogen used to generate this hybridoma was recombinant mouse TNF.



Expression of TNF by stimulated CD4+ and CD4- BALB/c spleen cells. Splenocytes from BALB/c mice were stimulated for 4 hours with PMA (5 ng/ml, Sigma, P-8139) and Ionomycin (500 ng, Sigma I-0634) in the presence of Brefeldin A (GolgiPlug, Cat. No. 555029). Cells were harvested, fixed, permeabilized and stained with APC-conjugated rat anti-mouse CD4 (APC-RM4-5, Cat. No. 553051) and either rat anti-mouse TNF antibody (PE-Cy7-MP6-XT22, Cat. No. 557644) (left panel) or immunoglobulin isotype control (PE-Cy7-R3-34, Cat. No. 557645), (right panel) by using BD Pharmingen staining protocol. To demonstrate specificity of staining the binding of PE-Cy7-MP6-XT22 was blocked by the preincubation of the conjugated antibody with molar excess of recombinant mouse TNF (0.25 μ g, Cat. No. 554589, data not shown) and by preincubation of the fixed/permeabilized cells with an excess of unlabelled MP6-XT22 antibody (5 μ g, Cat. No. 554416, data not shown) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence and isotype controls.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with PE-Cy7 under optimum conditions, and unconjugated antibody and free PE-Cy7 were removed.

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

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| Intracellular staining (flow cytometry) | Routinely Tested |
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Recommended Assay Procedure:

The PE-Cy7-conjugated MP6-XT22 antibody (Cat No. 557644) is available 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 ($\leq 0.5 \mu$ g mAb/million cells). For specific methodology, please visit the protocols section or chapter on intracellular staining for flow cytometry in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the conjugated MP6-XT22 antibody with a molar excess of ligand (e.g., recombinant mouse TNF; Cat No. 554589) prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabelled

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MP6-XT22 antibody (Cat. No. 554416) prior to staining. The staining technique and blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse and human cells is PE-Cy7-R3-34 (Cat. No. 557645). Use isotype controls at comparable concentrations to antibody of interest.

Suggested Companion Products

| Catalog Number | Name | Size | Clone |
|----------------|--|-----------|----------|
| 554416 | Purified Rat Anti-Mouse TNF | 0.1 mg | MP6-XT22 |
| 557645 | PE-Cy7™ Rat IgG1 κ Isotype Control | 0.1 mg | R3-34 |
| 555029 | Protein Transport Inhibitor (Containing Brefeldin A) | 1.0 ml | (none) |
| 555028 | BD Cytotfix/Cytoperm Plus Kit (with BD GolgiPlug) | 250 tests | (none) |
| 554652 | MiCK-1 Mouse 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. Please refer to wwwbdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at wwwbdbiosciences.com/colors.
4. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by 488-nm light and serves as an energy donor, coupled to the cyanine dye Cy7, which acts as an energy acceptor and fluoresces maximally at 780 nm. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. Although every effort is made to minimize the lot-to-lot variation in the efficiency of the fluorochrome energy transfer, differences in the residual emission from PE may be observed. Therefore, we recommend that individual compensation controls be performed for every PE-Cy7 conjugate. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light, and there is no significant overlap between PE-Cy7 and FITC emission spectra. When using dual-laser cytometers, which may directly excite both PE and Cy7, we recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
5. Warning: Some APC-Cy7 and PE-Cy7 conjugates show changes in their emission spectrum with prolonged exposure to formaldehyde. If you are unable to analyze fixed samples within four hours, we recommend that you use BD™ Stabilizing Fixative (Cat. No. 338036).
6. 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.
7. 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.
8. 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.
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References

- Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24. (Clone-specific)
- Hunter CA, Litton MJ, Remington JS, Abrams JS. Immunocytochemical detection of cytokines in the lymph nodes and brains of mice resistant or susceptible to toxoplasmic encephalitis. *J Infect Dis.* 1994; 170(4):939-945. (Clone-specific)
- Litton MJ, Sander B, Murphy E, O'Garra A, Abrams JS. Early expression of cytokines in lymph nodes after treatment in vivo with Staphylococcus enterotoxin B. *J Immunol Methods.* 1994; 175(1):47-58. (Clone-specific)
- 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)