Technical Data Sheet

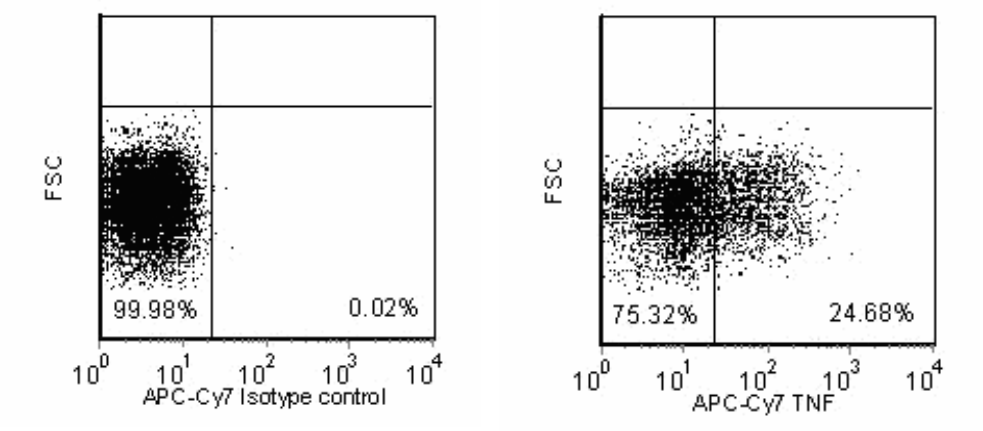
APC-Cy™7 Rat Anti-Mouse TNF

Product Information

Material Number:	**560658**
Size:	50 µg
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 protein stabilizer and ≤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.



Flow cytometric analysis for TNF in activated mouse splenocytes. *Mouse Intracellular Cytokine-1 positive control cells (MiCK-1) offered by BD Biosciences as MN 554652, are activated mouse splenocytes prepared in the presence of a protein transport inhibitor. Fixed and permeabilized MiCK-1 cells were stained either with a APC-Cy™7 Rat IgG1, κ isotype control (left panel) or with the APC-Cy™7 Rat Anti-Mouse 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 APC-Cy7 under optimum conditions, and unconjugated antibody and free APC-Cy7 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow cytometry: The MP6-XT22 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 mouse TNF (Cat. No. 554589) or (2) unlabeled MP6-XT22 antibody (Cat. No. 554416), prior to staining.

Cell Preparation: Investigators not wishing to utilize MiCK-1 cells may alternatively prepare mouse splenocytes (e.g BALB/c) stimulated for 4-6 hours with PMA (5 ng/mL, Sigma-Aldrich Cat. No. P-8139) and ionomycin (500 ng, Sigma-Aldrich Cat. No. I-0634) in the presence of 1 µg/mL Brefeldin A (BD GolgiPlug™ MN 555029). Investigators are advised to fix and permeabilize the cells prior to staining.

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Suggested Companion Products

Catalog Number	Name	Size	Clone
560534	APC-Cy™7 Rat IgG1, κ Isotype Control	0.1 mg	R3-34
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
554416	Purified Rat Anti-Mouse TNF	0.1 mg	MP6-XT22
554589	Recombinant Mouse TNF	10 µg	(none)
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)

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. 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).
4. 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.
5. This conjugated product is sold under license to the following patent: US Patent No. 5,714,386.
6. 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.
7. 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.
8. APC-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher.
9. APC-Cy7 is a tandem fluorochrome composed of Allophycocyanin (APC), which is excited by laser lines between 595 and 647 nm and serves as an energy donor, coupled to the cyanine dye Cy7™, which acts as an energy acceptor and fluoresces at 780 nm. BD Biosciences Pharmingen has maximized the fluorochrome energy transfer in APC-Cy7, thus maximizing its fluorescence emission intensity, minimizing residual emission from APC, and minimizing required electronic compensation in multilaser-laser flow cytometry systems. Note: Although every effort is made to minimize the lot-to-lot variation in residual emission from APC, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each APC-Cy7 conjugate.
10. 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.
11. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
12. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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