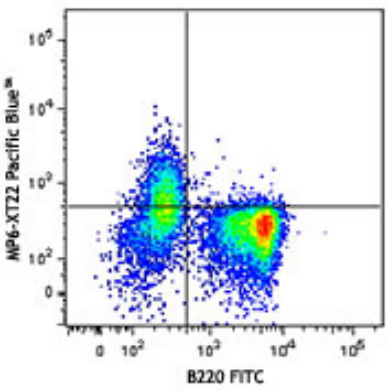
BioLegend
The path to legendary discovery™

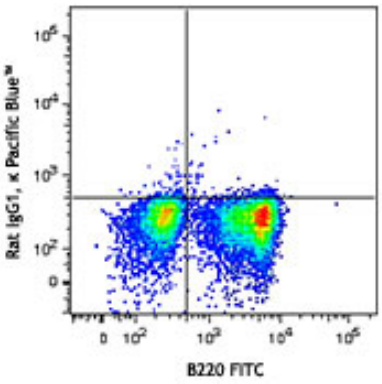
Product Data Sheet

Pacific Blue™ anti-mouse TNF-α

Catalog # / Size: 506318 / 100 µg
Clone: MP6-XT22
Isotype: Rat IgG1, κ
Immunogen: *E. coli*-expressed, recombinant mouse TNF-α
Reactivity: Mouse
Preparation: The antibody was purified by affinity chromatography, and conjugated with Pacific Blue™ under optimal conditions. The solution is free of unconjugated Pacific Blue™.
Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Concentration: 0.5 mg/ml
Storage: The antibody solution should be stored undiluted at 4°C and protected from prolonged exposure to light. **Do not freeze.**



PMA/Ionomycin-stimulated (6 hours) C57BL/6 mouse splenocytes surface stained with B220 FITC, and then intracellularly stained with TNF-&alpha (clone MP6-XT22) Pacific Blue™ (top) or rat IgG1, κ Pacific Blue™ isotype control (bottom).



Applications:

Applications: ICFC - *Quality tested*

Recommended Usage: Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For immunofluorescent staining, the suggested use of this reagent is ≤ 0.25 µg per 10^6 cells in 100 µl volume. It is recommended that the reagent be titrated for optimal performance for each application.

* Pacific Blue™ has a maximum emission of 455 nm when it is excited at 405 nm. Prior to using Pacific Blue™ conjugate for flow cytometric analysis, please verify your flow cytometer's capability of exciting and detecting the fluorochrome.
** Pacific Blue™ is a registered trademark of Molecular Probes, Inc. Pacific Blue™ dye antibody conjugates are sold under license from Molecular Probes, Inc. for research use only, except for use in combination with microarrays and high content screening, and are covered by pending and issued patents.

Application Notes: **ELISA or ELISPOT Detection:** The biotinylated MP6-XT22 antibody is useful as a detection antibody for a sandwich ELISA or ELISPOT assay, when used in conjunction with purified 6B8 antibody (Cat. No. 510802/510804) as the capture antibody.
Flow Cytometry[6,11,12]: The fluorochrome-labeled MP6-XT22 antibody is useful for intracellular immunofluorescent staining and flow cytometric analysis to identify TNF-α-producing cells within mixed cell populations. To view the intracellular cytokine staining protocol, please visit www.biolegend.com and click on the support section.
Neutralization[1,5,10]: The MP6-XT22 antibody can neutralize the bioactivity of natural or recombinant TNF-α. The LEAF™ purified antibody (Endotoxin <0.1 EU/µg, Azide-Free, 0.2 µm filtered) is recommended for neutralization of mouse TNF-α bioactivity *in vivo* and *in vitro* (Cat. No. 506310). For *in vivo* studies or highly sensitive assays, we recommend Ultra-LEAF™ purified antibody (Cat. No. 506332) with a lower endotoxin limit than standard LEAF™ purified antibodies (Endotoxin <0.01 EU/µg).
Additional reported applications (for the relevant formats) include: Western blotting, immunohistochemical staining of paraformaldehyde-fixed, saponin-treated frozen tissue sections[7-9], *in vivo* detection[5], immunofluorescence, and immunocytochemistry.
Note: For testing mouse TNF-α in serum, plasma or supernatant, BioLegend's ELISA Max™ Sets (Cat. No. 430901 to 430906) are specially developed and recommended.

Application References:
1. Abrams J, *et al.* 1992. *Immunol. Rev.* 127:5. (Neut)
2. Abrams J, *et al.* 1995. *Curr. Prot. Immunol.* John Wiley and Sons, New York. Unit 6.20
3. Mo X, *et al.* 1995. *J. Virol.* 69:1288.
4. Sarawar S, *et al.* 1994. *J. Immunol.* 153:1246.
5. Via C, *et al.* 2001. *J. Immunol.* 167:6821. (Neut)

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6. Infante-Duarte C, *et al.* 2000 *J. Immunol.* 165:6107. (FC)
7. Jacobs M, *et al.* 2000. *Immunology* 100:494. (IHC)
8. Marinova-Mutachieva L, *et al.* 1997. *Clin. Exp. Immunol.* 107:507. (IHC)
9. Williams RO, *et al.* 2000. *J. Immunol.* 165:7240. (IHC)
10. Scanga CA, *et al.* 1999. *Infect. Immun.* 67:4531. (Neut)
11. Akilov OE, *et al.* 2007. *J. Leukoc. Biol.* 2007;10.1189/jlb.0706439. (FC)
12. Lawson BR, *et al.* 2007. *J. Immunol.* 178:5366. (FC)
13. Patole PS, *et al.* 2005. *J. Am. Soc. Nephrol.* 16:3273. PubMed
14. Wu S, *et al.* 2005. *Neurosci Lett.* 394:158. PubMed
15. Carlson MJ, *et al.* 2009. *Blood* 113:1365. PubMed

Description: TNF-α is secreted by macrophages, monocytes, neutrophils, T-cells (principally $CD4^+$), and NK-cells. Many transformed cell lines also secrete TNF-α. Monomeric mouse TNF-α is a 156 amino acid protein (N-glycosylated) with a reported molecular weight of 17.5 kD. TNF-α forms multimeric complexes; stable trimers are most common in solution. A 26 kD membrane form of TNF-α has also been described. TNF-α binding to surface receptors elicits a wide array of biologic activities including: cytolysis and cytostasis of many tumor cell lines *in vitro*, hemorrhagic necrosis of tumors *in vivo*, increased fibroblast proliferation, and enhanced chemotaxis and phagocytosis in neutrophils.

Antigen References:

1. Fitzgerald K, *et al.* Eds. 2001. The Cytokine FactsBook. Academic Press, San Diego.
2. Beutler B, *et al.* 1988. *Annu. Rev. Biochem.* 57:505.
3. Beutler B, *et al.* 1989. *Annu. Rev. Immunol.* 7:625.
4. Tracey K, *et al.* 1993. *Crit. Care Med.* 21:S415.

Related Products:

Product	Clone	Application
Cell Staining Buffer		FC, ICC, ICFC
Fixation Buffer		ICC, ICFC
Permeabilization Wash Buffer (10X)		ICC, ICFC, IHC
Brefeldin A Solution (1,000X)		ICFC
Monensin Solution (1,000X)		ICFC
7-AAD Viability Staining Solution		FC
Pacific Blue™ Rat IgG1, κ Isotype Ctrl	RTK2071	FC, ICFC

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