SONY

Product Data Sheet

Alexa Fluor® 488 anti-mouse TNF-α

Catalog # / Size: 3131575 / 25 µg
3131565 / 100 µg

Clone: MP6-XT22

Isotype: Rat IgG1, κ

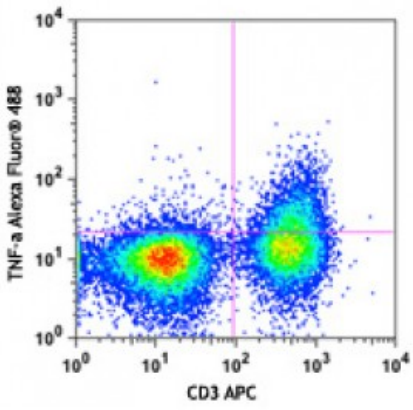
Immunogen: *E. coli*-expressed, recombinant mouse TNF-α

Reactivity: Mouse

Preparation: The antibody was purified by affinity chromatography, and conjugated with Alexa Fluor® 488 under optimal conditions.

Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.

Concentration: 0.5



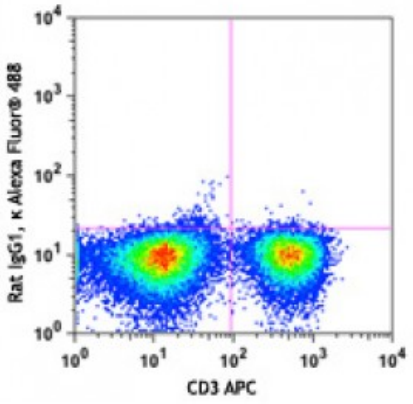
PMA + Ionomycin-stimulated C57BL/6 mouse splenocytes (in the presence of monensin) were stained with CD3 APC, fixed, permeabilized and then stained with TNF-α (clone MP6-XT22) Alexa Fluor® 488 (top) or rat IgG1, κ Alexa Fluor® 488 iso

Applications:

Applications: Flow Cytometry

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

* Alexa Fluor® 488 has a maximum emission of 519 nm when it is excited at 488 nm.



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.

Neutralization[1,5,10,16,17]: 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/microg).
Additional reported applications (for the relevant formats) include: Western blotting, immunohistochemical staining of paraformaldehyde-fixed, saponin-treated frozen tissue sections[7-9], *in vivo* detection5, 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)
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.