SONY

Product Data Sheet

Brilliant Violet 750™ anti-mouse TNF-α

Catalog # / Size: 3131790 / 50 µg

Clone: MP6-XT22

Isotype: Rat IgG1, κ

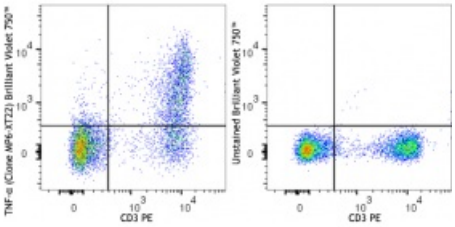
Immunogen: E. coli-expressed, recombinant mouse TNF-α

Reactivity: Mouse

Preparation: The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 750™ under optimal conditions. The solution is free of unconjugated Brilliant Violet 750™ and unconjugated antibody.

Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA).

Concentration: 0.2 mg/ml



PMA + Ionomycin-stimulated C57BL/6 mouse splenocytes (in the presence of Brefeldin A) were stained with CD3 PE, fixed, permeabilized and then stained with (left) or without (right) TNF-α (clone MP6-XT22) Brilliant Violet 750™.

Applications:

Applications: Intracellular Flow Cytometry

Recommended Usage: Each lot of this antibody is quality control tested by intracellular flow cytometry using our True-Phos™ Perm Buffer in Cell Suspensions Protocol. For flow cytometric staining, the suggested use of this reagent is ≤ 0.5 µg per million cells in 100 µl volume. It is recommended that the reagent be titrated for optimal performance for each application.

Brilliant Violet 750™ excites at 405 nm and emits at 750 nm. The bandpass filter 780/60 nm is recommended for detection, although filter optimization may be required depending on other fluorophores used. Be sure to verify that your cytometer configuration and software setup are appropriate for detecting this channel. Refer to your instrument manual or manufacturer for support. Brilliant Violet 750™ is a trademark of Sirigen Group Ltd.

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 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.

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.

Application References:

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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
16. Shivakumar P, *et al.* 2017. *JCI Insight.* 2:e88747 1. PubMed
17. Kearney CJ*, et al.* 2017. *Cell Death Differ*. 10.1038/cdd.2017.94. PubMed

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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.