SONY

Product Data Sheet

Brilliant Violet 711™ anti-human TNF-α

Catalog # / Size: 3114695 / 25 tests
3114700 / 100 tests

Clone: MAb11

Isotype: Mouse IgG1, κ

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

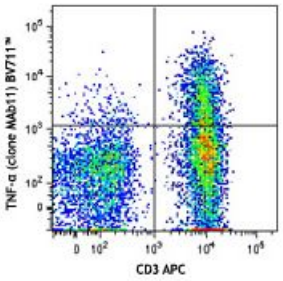
Reactivity: Human, Non-human primate, Other

Preparation: The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 711™ under optimal conditions.

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

Workshop Number: IV T-164

Concentration: Lot-specific



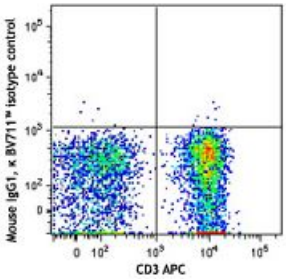
PMA+ ionomycin-stimulated (6 hours) human peripheral blood lymphocytes (in the presence of monensin) were stained with CD3 APC, fixed, permeabilized, and then stained with TNF-α (clone MAb11) Brilliant Violet 711™ (top) or mouse IgG1, κBrilliant Violet 711™ isotype control (bottom).

Applications:

Applications: Flow Cytometry, Intracellular Staining for 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 ≤5 µl per million cells or 5 µl per 100 µl of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.

Brilliant Violet 711™ excites at 405 nm and emits at 711 nm. The bandpass filter 710/50 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 711™ is a trademark of Sirigen Group Ltd.



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Application Notes:

Other or ELISPOT Detection: The biotinylated MAb11 antibody is useful as the detection antibody in a sandwich Other or ELISPOT, when used in conjunction with the purified MAb1 antibody (Cat. No. 502802/502804) as the capture antibody.

Flow Cytometry[3,5,6,10]: The fluorochrome-labeled MAb11 antibody is useful for intracellular and membrane-bound immunofluorescent staining and flow cytometric analysis to identify TNF-α-producing cells within mixed cell populations.

Additional reported applications (for the relevant formats) include: neutralization[1,2], immunohistochemical staining of paraformaldehyde-fixed, saponin-treated frozen tissue sections4 and acetone-fixed frozen tissue sections[8], immunocytochemistry[7], and immunofluorescence[9]. The MAb11 antibody can neutralize the bioactivity of natural or recombinant TNF-α.

Note: For testing human TNF-α in serum or plasma, Other MAX™ Sets (Cat. No. 430201 to 430206) are specially developed and recommended. The LEAF™ purified antibody (Endotoxin <0.1 EU/µg, Azide-Free, 0.2 µm filtered) is recommended for neutralization of human TNF-α bioactivity (Cat. No. 502922).

The Purified MAb1 antibody is useful in neutralization2 and as the capture antibody in a sandwich Other or ELISPOT assay, when used in conjunction with the biotinylated MAb11 antibody (Cat. No. 502904/502914) as the detecting antibody.

Application References:

1. Rathjen D, *et al.* 1991. *Mol. Immunol.* 28:79. (Neut)
2. Ablamunits V, *et al*. 2010. *Eur. J. Immunol*. 40:2891. (Neut)
3. Enr quez J, *et al.* 2002. *Adv. Perit. Dial.* 18:177. (ICFC)
4. Andersson U, *et al.* 1999. *Detection and quantification of gene expression*. New York:Springer-Verlag. (IHC)
5. Chen H, *et al.* 2005. *J. Immunol.* 175:591. (ICFC)
6. Iwamoto S, *et al.* 2007. *J. Immunol.* 179:1449. (ICFC) PubMed
7. Andersson U, *et al.* 2000. *J. Exp. Med.* 192:565. (ICC)
8. Moormann AM, *et al.* 1999. *J. Infect. Dis.* 180:1987. (IHC)
9. Zhao XJ, *et al.* 2003. *J. Immunol.* 170:2923. (IF)
10. Rieger R, *et al.* 2009. *Cancer Gene Ther.* 1:53-64. (FC)
11. Maksaereekul S, *et al.* 2009. *Vaccine*. 28:3754 (FC)

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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 human TNF-α is a 157 amino acid protein (non-glycosylated) with a reported molecular weight of 17 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 biological activities including: cytolysis and cytostasis of many tumor cell lines *in vitro*, hemorraghic 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.

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