

**Brilliant Violet 650™ anti-human TNF-α**

**Catalog # / Size:** 3114685 / 25 tests  
3114690 / 100 tests

**Clone:** MAb11

**Isotype:** Mouse IgG1, κ

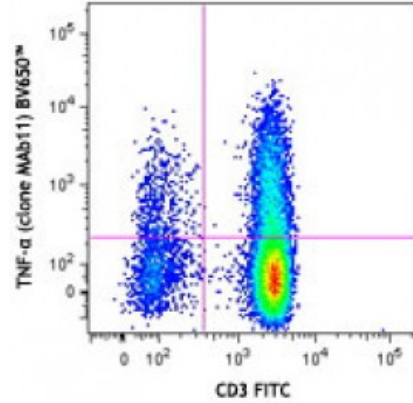
**Immunogen:** *E. coli*-expressed, recombinant human TNF-α

**Reactivity:** Human

**Preparation:** The antibody was purified by affinity chromatography and conjugated with Brilliant Violet 650™ under optimal conditions. The solution is free of unconjugated Brilliant Violet 650™ and unconjugated antibody.

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

**Concentration:** Lot-specific

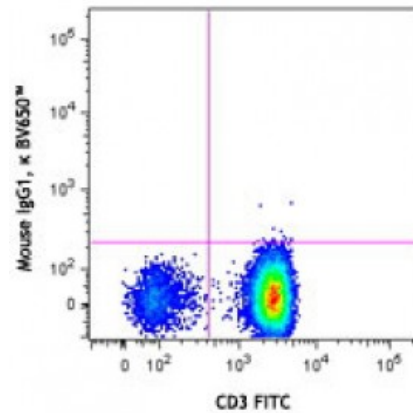


PMA+ionomycin-stimulated (6 hours) human peripheral blood lymphocytes (in the presence of monensin) were stained with CD3 FITC, fixed, permeabilized, and then stained with TNF-α (clone MAb11) Brilliant Violet 650™ (top) or mouse IgG1, κ

**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 ≤5 microL per million cells or 5 microL per 100 microL of whole blood. It is recommended that the reagent be titrated for optimal performance for each application.



Brilliant Violet 650™ excites at 405 nm and emits at 645 nm. The bandpass filter 660/20 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 650™ is a trademark of Sirigen Group Ltd.

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buyer a non-transferable right to use the purchased product for research purposes only. This product may not be resold or incorporated in any manner into another product for resale. Any use for therapeutics or diagnostics is strictly prohibited. This product is covered by U.S. Patent(s), pending patent applications and foreign equivalents.

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

**Flow Cytometry<sup>3,5,6,10</sup>:** The fluorochrome-labeled MAb11 antibody is useful for intracellular and membrane-bound immunofluorescent staining and flow cytometric analysis to identify TNF- $\alpha$ -producing cells within mixed cell populations.

**Additional reported applications (for the relevant formats) include:** neutralization<sup>1,2</sup>, immunohistochemical staining of paraformaldehyde-fixed, saponin-treated frozen tissue sections<sup>4</sup> and acetone-fixed frozen tissue sections<sup>8</sup>, immunocytochemistry<sup>7</sup>, and immunofluorescence<sup>9</sup>. The MAb11 antibody can neutralize the bioactivity of natural or recombinant TNF- $\alpha$ .

**Note:** For testing human TNF- $\alpha$  in serum or plasma, BioLegend's ELISA Max™ Sets (Cat. No. 430201 to 430206) are specially developed and recommended. The LEAF™ purified antibody (Endotoxin <0.1 EU/ $\mu$ g, Azide-Free, 0.2  $\mu$ m filtered) is recommended for neutralization of human TNF- $\alpha$  bioactivity (Cat. No. 502922).

The Purified MAb1 antibody is useful in neutralization<sup>2</sup> and as the capture antibody in a sandwich ELISA 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. Danis V, *et al.* 1991. *Clin. Exp. Immunol.* 85:143. (Neut)
  3. Enrquez 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)

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**Description:** TNF- $\alpha$  is secreted by macrophages, monocytes, neutrophils, T cells (principally

CD4<sup>+</sup>), and NK cells. Many transformed cell lines also secrete TNF- $\alpha$ . Monomeric human TNF- $\alpha$  is a 157 amino acid protein (non-glycosylated) with a reported molecular weight of 17 kD. TNF- $\alpha$  forms multimeric complexes; stable trimers are most common in solution. A 26 kD membrane form of TNF- $\alpha$  has also been described. TNF- $\alpha$  binding to surface receptors elicits a wide array of biological 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.