Technical Data Sheet

BV650 Rat Anti-Mouse TNF

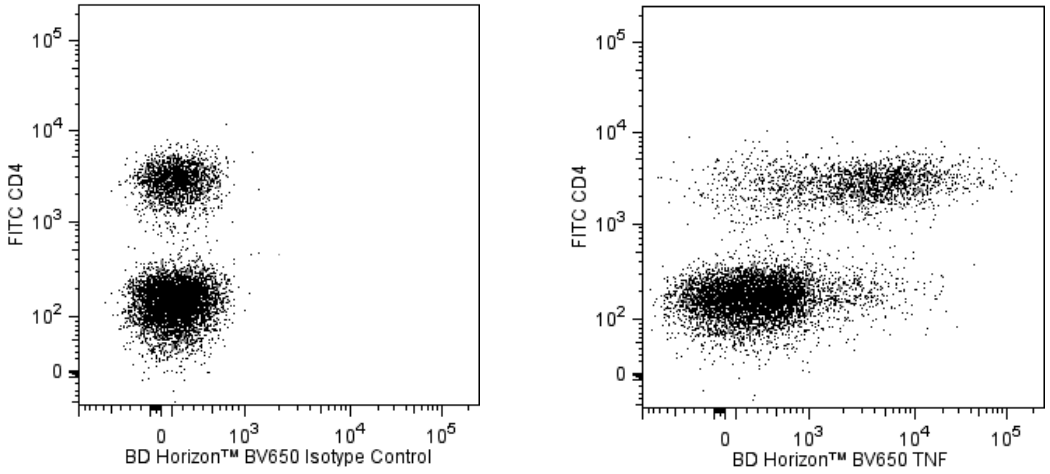
Product Information

Material Number:	**563943**
Alternate Name:	Tnf; Tnfa; TNF alpha; TNF-a; Tnfsf1a; Tnfsf2; TNFSF2; Cachectin; DIF
Size:	50 µg
Concentration:	0.2 mg/ml
Clone:	MP6-XT22
Immunogen:	Recombinant Mouse TNF
Isotype:	Rat IgG1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The MP6-XT22 antibody specifically binds to mouse Tumor Necrosis Factor (TNF, also known as TNF-α). TNF is produced by many activated cell types including monocytes, macrophages, astrocytes, granulocytes, mast cells, T and B lymphocytes, NK cells, keratinocytes, fibroblasts, adipocytes, and certain tumor cells. Activated cells express type II transmembrane TNF glycoproteins that associate as homotrimeric complexes. After enzymatic cleavage, the extracellular regions of membrane TNF are shed as soluble homotrimers. TNF is a potent multifunctional cytokine that can exert regulatory and cytotoxic effects on a wide range of normal lymphoid and non-lymphoid cells and tumor cells. Although TNF serves as a primary mediator in protective immune responses against microbial and viral pathogens, it can also drive systemic pathophysiologic responses including septic shock, cachexia and autoimmune diseases. Mouse TNF exerts its biological activities by binding and signaling through cell surface membrane Type I and Type II TNF Receptors (aka, TNFRI/CD120a and TNFRII/CD120b, respectively).

The antibody was conjugated to BD Horizon™ BV650 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. This dye is a tandem fluorochrome of BD Horizon™ BV421 with an Ex Max of 405-nm and an acceptor dye with an Em Max at 650-nm. BD Horizon™ BV650 can be excited by the violet laser and detected in a filter used to detect APC-like dyes (eg, 660/20-nm filter). Due to the excitation and emission characteristics of the acceptor dye, there will be spillover into the APC and Alexa Fluor® 700 detectors. However, the spillover can be corrected through compensation as with any other dye combination.



Two color flow cytometric analysis of TNF expression by stimulated mouse splenocytes. *Mouse splenic leucocytes were stimulated for 5 hours with Phorbol 12-Myristate 13-Acetate (PMA; Sigma P-8139; 50 ng/ml) and Ionomycin (Sigma I-0634; 1 µg/ml) in the presence of BD GolgiStop™ Protein Transport Inhibitor (containing Monensin) (Cat. No. 554724). The cells were harvested and washed with stain buffer and fixed and permeabilized with BD Cytofix/Cytoperm™ Fixation and Permeabilization Solution (Cat. No. 554722). The cells were then washed and stained in BD Perm/Wash™ Buffer (Cat. No. 554723) with FITC Rat Anti-Mouse CD4 antibody (Cat. No. 553047/553046/561835) and either BD Horizon™ BV650 Rat IgG1, κ Isotype Control (Cat. No. 563848; Left Panel) or BD Horizon™ BV650 Rat Anti-Mouse TNF antibody (Cat. No. 563943; Right Panel) by using BD Biosciences Intracellular Cytokine Staining protocol. Flow cytometric dot plots showing correlated expression of TNF (or Ig Isotype control staining) versus CD4 were derived from gated events with the forward and side light-scatter characteristics of intact stimulated leucocytes. Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon™ BV650 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV650 were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554657	Stain Buffer (BSA)	500 ml	(none)
563848	BV650 Rat IgG1, κ Isotype Control	50 µg	R3-34
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554722	Fixation and Permeabilization Solution	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
553047	FITC Rat Anti-Mouse CD4	0.5 mg	RM4-5
553046	FITC Rat Anti-Mouse CD4	0.1 mg	RM4-5
561835	FITC Rat Anti-Mouse CD4	25 µg	RM4-5

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
5. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
6. Brilliant Violet™ 650 is a trademark of Sirigen.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Abrams J. Immunoenzymetric assay of mouse and human cytokines using NIP-labeled anti-cytokine antibodies. *Curr Protoc Immunol.* 2001; 1:6.20-6.21. (Clone-specific: ELISA)

Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anti-cytokine monoclonal antibody development: immunoassay of IL-10 and IL-5 in clinical samples. *Immunol Rev.* 1992; 127:5-24. (Clone-specific: Blocking, ELISA)

Bitsaktsis C, Winslow G. Fatal recall responses mediated by CD8 T cells during intracellular bacterial challenge infection. *J Immunol.* 2006; 177(7):4644-4651. (Clone-specific: Blocking)

Hunter CA, Litton MJ, Remington JS, Abrams JS. Immunocytochemical detection of cytokines in the lymph nodes and brains of mice resistant or susceptible to toxoplasmic encephalitis. *J Infect Dis.* 1994; 170(4):939-945. (Clone-specific: Immunohistochemistry)

Litton MJ, Sander B, Murphy E, O'Garra A, Abrams JS. Early expression of cytokines in lymph nodes after treatment in vivo with Staphylococcus enterotoxin B. *J Immunol Methods.* 1994; 175(1):47-58. (Clone-specific: Immunohistochemistry)

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128. (Methodology: Flow cytometry, IC/FCM Block)

Yang J, Kawamura I, Zhu H, Mitsuyama M. Involvement of natural killer cells in nitric oxide production by spleen cells after stimulation with Mycobacterium bovis BCG. Study of the mechanism of the different abilities of viable and killed BCG. *J Immunol.* 1995; 155(12):5728-5735. (Clone-specific: Blocking)

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