Technical Data Sheet

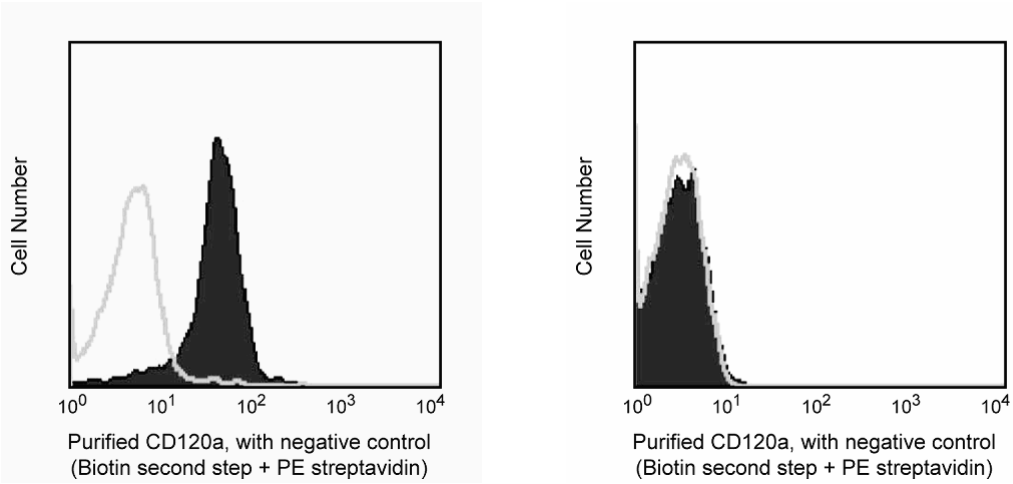
Purified Mouse Anti-Human CD120a

Product Information

Material Number:	**550514**
Alternate Name:	TNF Receptor Type I
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	MABTNFR1-B1
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The TNFR1-B1 antibody reacts with the extracellular domain of CD120A which is also known as the 55 kDa receptor for the human cytokines, tumor necrosis factor (TNF) and lymphotoxin-alpha (LT-α). This receptor is referred to as the p55 or Type I Tumor Necrosis Factor Receptor (TNFRI). TNFRI are expressed by a variety of cell lines, tumor cells, and normal cell types including T cells, monocytes, macrophages, neutrophils, endothelial cells, hepatocytes, chondrocytes, and fibroblasts. Naive B cells express very low or undetectable levels of TNFRI whereas mature erythrocytes and platelets are uniformly negative for TNFRI expression. For the generation of the MABTNFR1-B1 hybridoma, BALB/c mice were immunized with purified, full-length human TNFRI protein that was expressed by insect cells that were infected with a recombinant human TNFRI-baculovirus expression vector. MABTNFR1-B1 specifically binds to natural and recombinant truncated forms of TNFRI.



Expression of cell surface TNFRI by non-activated human PBMC or RBC. *Human PBMC (left panel) and red blood cells (right panel) isolated by density centrifugation (Ficoll-Paque™) were stained with MABTNFR1-B1 (0.5 µg, Cat No. 550514) followed by biotinylated goat anti-mouse Ig (0.25 µg, Cat . No. 553999) and Streptavidin Phycoerythrin (0.015 µg, Cat. No. 554061) in a three-step staining protocol to amplify immunofluorescent signals. Staining with the MABTNFR1-B1 antibody (filled histograms) is compared to staining obtained using the secondary antibody alone (open histograms). Histograms in the left panel are gated on the lymphocyte population. Note: Certain human cell lines or cell types (e.g., neutrophils, monocytes) can first be treated with reagents that block receptors for the Fc regions of immunoglobulin to avoid nonspecific immunofluorescent staining mediated by Fc receptors.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4° C.

Application Notes

Application

Flow cytometry	Routinely Tested

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Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The purified form of MABTNFR1-B1 (Cat. No. 550514) can be used for the immunofluorescent staining (≤ 1 µg antibody/10e6 cells) and flow cytometric analysis of normal human lymphoid cells or cell lines to measure their expressed levels of surface TNFRI. An appropriate purified isotype control antibody is clone G155-178 (Cat. No. 555571). A three-step staining protocol is recommended for maximizing the detection of TNFRI expressed by cells as detailed in the figure legend (see below). As a demonstration of specificity, the binding of MABTNFR1-B1 to TNFRI is inhibited when human TNFRI+ target cells are preincubated with saturating levels of recombinant human TNF at 4°C, i.e., when the TNFRI are bound with ligand. Based on our data, recombinant human TNF at ≥ 50 ng/10e6 cells is sufficient to completely inhibit the binding of MABTNFR1-B1 (0.06 µg/10e6 cells). Please note that as a consequence of in vivo or in vitro activation, cell surface TNFRI can either be shed by cells or transiently expressed at higher levels. As a result, cellular activation can affect the cell's overall expressed level of surface TNFRI.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555571	Purified Mouse IgG2a, κ Isotype Control	0.1 mg	G155-178
553999	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	0.5 mg	Polyclonal
554061	PE Streptavidin	0.5 mg	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. Ficoll-Paque is a trademark of Amersham Biosciences Limited.

References

Aggarwal S, Gollapudi S, Gupta S. Increased TNF-alpha-induced apoptosis in lymphocytes from aged humans: changes in TNF-alpha receptor expression and activation of caspases. *J Immunol.* 1999; 162(4):2154-2161.(Biology)

Brockhaus M, Schoenfeld HJ, Schlaeger EJ, Hunziker W, Lesslauer W, Loetscher H. Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies. *Proc Natl Acad Sci U S A.* 1990; 87(8):3127-3131.(Biology)

Browning JL, Dougas I, Ngam-ek A, et al. Characterization of surface lymphotoxin forms. Use of specific monoclonal antibodies and soluble receptors. *J Immunol.* 1995; 154(1):33-46.(Biology)

Erikstein BK, Smeland EB, Blomhoff HK, et al. Independent regulation of 55-kDa and 75-kDa tumor necrosis factor receptors during activation of human peripheral blood B lymphocytes. *Eur J Immunol.* 1991; 21(4):1033-1037.(Biology)

Gehr G, Gentz R, Brockhaus M, Loetscher H, Lesslauer W. Both tumor necrosis factor receptor types mediate proliferative signals in human mononuclear cell activation. *J Immunol.* 1992; 149(3):911-917.(Biology)

Heilig B, Mapara M, Brockhaus M, Krauth K, Dörken B. Two types of TNF receptors are expressed on human normal and malignant B lymphocytes. *Clin Immunol Immunopathol.* 1991; 61(2):260-267.(Biology)

Hohmann HP, Remy R, Brockhaus M, van Loon AP. Two different cell types have different major receptors for human tumor necrosis factor (TNF alpha). *J Biol Chem.* 1989; 264(25):14927-14934.(Biology)

Munker R, DiPersio J, Koeffler HP. Tumor necrosis factor: receptors on hematopoietic cells. *Blood.* 1987; 70(6):1730-1734.(Biology)

Wallach D, Engelmann H, Nophar Y, et al. Soluble and cell surface receptors for tumor necrosis factor. *Agents Actions Suppl.* 1991; 35:51-57.(Biology)

Zola H. Detection of cytokine receptors by flow cytometry. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W, ed. *Current Protocols in Immunology.* New York: Green Publishing Associates and Wiley-Interscience; 1995:6.21.1-6.21.18.(Biology)