

Technical Data Sheet

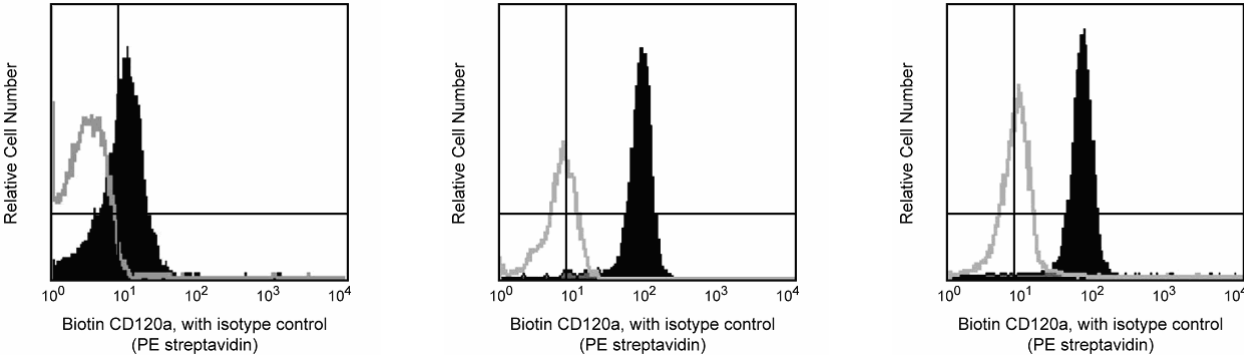
Biotin Mouse Anti-Human CD120a

Product Information

Material Number:	550900
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 (TNFR). TNFR1 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 TNFR1 whereas mature erythrocytes and platelets are uniformly negative for TNFR1 expression. For the generation of the MABTNFR1-B1 hybridoma, BALB/c mice were immunized with purified, full-length human TNFR1 protein that was expressed by insect cells that were infected with a recombinant human TNFR1-baculovirus expression vector. MABTNFR1-B1 specifically binds to natural and recombinant truncated forms of TNFR1.



Expression of cell surface TNFR1 by whole-lysed human blood. Whole human blood was stained with biotinylated MABTNFR1-B1 (0.125 µg, Cat No. 550900) followed by streptavidin phycoerythrin (0.015 µg, Cat. No. 554061). Red blood cells were subsequently lysed with PharmLyse™ Lysing Buffer (Cat No. 555899). Staining with the MABTNFR1-B1 antibody (filled histogram) is compared to staining obtained using the isotype control antibody (open histograms). Histograms in figure are gated on the lymphocyte (left panel), monocyte (middle panel) and granulocyte (right panel) populations based on the side and forward light scatter characteristics. 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 (for example see Reference by Browning et. al.).

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with biotin under optimum conditions, and unreacted biotin was removed. Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Flow cytometry	Routinely Tested
ELISA Detection	Tested During Development

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The biotinylated form of MABTNFR1-B1 (Cat. No. 550900) can be used for the immunofluorescent staining (≤ 1 µg antibody/10e6 cells) and flow cytometric analysis of nucleated human cells to measure their expressed levels

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of surface TNFRI. A two-layer staining protocol is recommended for maximizing the detection of TNFRI expressed by cells as detailed in the figure legend. 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 levels above 50 ng/10e6 cells is sufficient to completely inhibit the binding of MABTNFR1-B1 (0.06 µg/10e6 cells). Please note also 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.

ELISA Detection: Biotinylated MABTNFR1-B1 (Cat. No. 552536) serves as the detection antibody in a sandwich ELISA for measuring human soluble TNFRI protein levels. Biotin MABTNFR1-B1 antibody can be paired with the purified MABTNFR1-A1 anti-human TNFRI (Cat No. 552535) using recombinant human soluble TNFRI as the standard. This detection antibody should be titrated between 0.5 - 1 µg/ml to determine its optimal concentration for ELISA detection. To obtain linear standard curves, doubling dilutions of recombinant soluble human TNFRI ranging from 2000 to 15 pg/ml are recommended for inclusion in each ELISA plate. For specific methodology please visit the online protocols sections or the chapter on ELISA in the Immune Functions Handbook, both of which are posted on our web site, www.bdbiosciences.com.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553455	Biotin Mouse IgG2a, κ Isotype Control	0.25 mg	G155-178
554061	PE Streptavidin	0.5 mg	(none)
555899	Lysing Buffer	100 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharming/en/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.

References

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