

Technical Data Sheet

Biotin Mouse Anti-Human CD120a

Product Information

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| Material Number: | 552536 |
| 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 $\leq 0.09\%$ 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 (TNFR1). 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.

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

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| ELISA Detection | Routinely Tested |
| Flow cytometry | Routinely Tested |

Recommended Assay Procedure:

ELISA Detection: Biotinylated MABTNFR1-B1 (Cat. No. 552536) serves as the detection antibody in a sandwich ELISA for measuring human soluble TNFR1 protein levels. Biotin MABTNFR1-B1 antibody can be paired with the purified MABTNFR1-A1 anti-human TNFR1 (Cat No. 552535) using recombinant human soluble TNFR1 as the standard. This detection antibody should be titrated between 0.5 - 1 $\mu\text{g/ml}$ to determine its optimal concentration for ELISA detection. To obtain linear standard curves, doubling dilutions of recombinant soluble human TNFR1 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.

Note: This ELISA antibody pair shows no cross-reactivity with the following recombinant human cytokines: IL-1RII, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, TNF, TNF- β , sTNFRII, GM-CSF, TGF β . This ELISA antibody pair also shows no cross-reactivity with recombinant cytokines from other species: rat TNF, mouse TNF, mouse sTNFR1, mouse sTNFRII.

Immunofluorescent Staining and Flow Cytometric Analysis: The biotinylated form of MABTNFR1-B1 can be used for the immunofluorescent staining ($\leq 1 \mu\text{g}$ antibody/10e6 cells) and flow cytometric analysis of nucleated human cells to measure their expressed levels of surface TNFR1. An appropriate biotinylated immunoglobulin isotype control is clone G155-178 (Cat. No. 551074). A two-layer staining protocol is recommended for maximizing the detection of TNFR1 expressed by cells. As a demonstration of specificity, the binding of MABTNFR1-B1 to TNFR1 is inhibited when human TNFR1+ target cells are pre-incubated with saturating levels of recombinant human TNF at 4°C, i.e., when the TNFR1 are bound with ligand. Based on our data, recombinant human TNF at levels above 50 $\text{ng}/10\text{e}6$ cells is sufficient to completely inhibit the binding of MABTNFR1-B1 (0.06 $\mu\text{g}/10\text{e}6$ cells). Please note also that as a consequence of in vivo or in vitro activation, cell surface TNFR1 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 TNFR1.

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Suggested Companion Products

| <u>Catalog Number</u> | <u>Name</u> | <u>Size</u> | <u>Clone</u> |
|-----------------------|--|-------------|--------------|
| 552535 | Purified Mouse Anti-Human CD120a | 0.5 mg | MABTNFR1-A1 |
| 554061 | PE Streptavidin | 0.5 mg | (none) |
| 551074 | Biotin Mouse IgG2a, κ Isotype Control | 0.25 mg | G155-178 |

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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