Technical Data Sheet

Purified Hamster Anti-Mouse CD120b

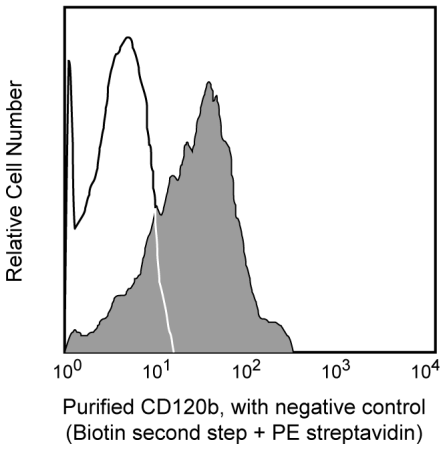
Product Information

Material Number:	**559916**
Alternate Name:	TNF Receptor, Type II/p75
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	TR75-89
Immunogen:	Mouse Type II TNFR
Isotype:	Armenian Hamster IgG1, λ3
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The TR75-89 antibody reacts with the extracellular region of CD120b, the 75 kDa receptor for the mouse cytokines, tumor necrosis factor (TNF, aka TNF-α) and lymphotoxin-alpha (LT-α, aka lymphotoxin, TNF-β). This receptor, referred to as the p75 or Type II Tumor Necrosis Factor Receptor (TNFRII), or TNFRSF1B, is expressed by a variety of cell lines and normal cell types including T cells, monocytes, macrophages, and neutrophils. Resting B cells express low or undetectable levels of TNFRII whereas mature erythrocytes are uniformly negative for TNFRII expression. In addition, the TR75-89 antibody can bind to a soluble, truncated form of the mouse Type II TNFR that is shed by cells in response to certain stimuli, e.g., cells treated with LPS or TNF. The in vivo administration of nonblocking, nonagonistic TR75-89 antibody reportedly results in the linear accumulation of shed, soluble forms of p75 TNFR in the circulation. The TR75-89 antibody does not recognize the 55 kDa (p55) Type I TNFR (aka, CD120a). TR75-89 does not block the binding and does not neutralize the bioactivity of the TNF ligand on L929 target cell populations that express p75 (and p55) TNFR. The immunogen used to generate the TR75-89 hybridoma was a purified, soluble extracellular domain of the mouse Type II TNFR.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Expression of cell surface p75 TNFR by BALB/c lymph node T cells. *BALB/c lymph node cells were preincubated (~15 min., 4°C) with purified 2.4G2 antibody [rat anti-mouse CD16 (FcγIII)/CD32 (FcγII); Cat. No. 553142; 1 µg antibody/10e6 cells] to block Fc receptor-mediated nonspecific staining by staining antibodies. The cells were stained (30 min., 4°C) with purified TR75-89 antibody (0.5 µg mAb/10e6 cells; Cat. No. 559916). The cells were washed and were incubated (30 min., 4°C) with a biotin-conjugated cocktail of mouse anti-hamster antibodies (Clones G70204 + G94-56; Cat. No. 554010; 0.5 µg mAb cocktail/10e6 cells). Finally, the cells were washed, were incubated with R-PE-conjugated streptavidin (Cat. No. 554061; 0.015 µg PE-SA/10e6 cells) and FITC-RA3-6B2/B220 (Cat. No. 553088; 0.06 µg mAb/10e6 cells), and were washed in preparation for flow cytometric analysis with a FACScan™ Flow Cytometer. The immunofluorescent staining patterns for cells stained in the first stage with either purified TR75-89 antibody (filled histogram) or no antibody (background staining; empty histogram) followed by the 2nd- and 3rd-layer reagents are shown. The histograms were generated from reanalyzed flow cytometric data files that were gated for FITC-negative events with the light-scattering characteristics of lymphocytes, i.e., B220-negative lymph node T cells.*

Preparation and Storage

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

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Application Notes

Application

Flow cytometry	Routinely Tested
Immunoprecipitation	Reported

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The purified form of TR75-89 (Cat. No. 559916) can be used for the immunofluorescent staining (≤ 1 µg antibody/10e6 cells) and flow cytometric analysis of normal mouse cells or cell lines to measure their expressed levels of p75 TNFR. An appropriate purified immunoglobulin isotype control is clone G235-2356 (Cat. No. 553951). A three-layer staining protocol is recommended for maximizing the detection of p75 TNFR expressed by cells as detailed in the figure legend.

Note: TR75-89 is a nonblocking antibody that can be used for the unobstructed immunofluorescent staining and flow cytometric analysis of cells in systems where ligands (e.g., TNF) for p75 TNF receptors are present. Based on our testing results (data not shown), the presence of exogenous recombinant mouse TNF (Cat. No. 554589) at levels ≤ 1 µg per 10e6 cells was insufficient to inhibit the binding of TR75-89 to L929 cells that express p75 TNFR (at 0.25 µg antibody/10e6 cells). Please note also that as a consequence of in vivo or in vitro activation, cell surface p75 TNFR 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 p75 TNFR.

IP: The TR75-89 antibody has been reported to be useful for the immunoprecipitation of p75 TNFR from lysates of mouse Meth A fibrosarcoma cells. Please note that this application is not routinely tested at BD Biosciences Pharmingen.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553951	Purified Hamster IgG Isotype Control	0.5 mg	G235-2356
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)	0.5 mg	2.4G2
554010	Biotin Mouse Anti-Armenian and Syrian Hamster IgG Cocktail	0.5 mg	(none)
554061	PE Streptavidin	0.5 mg	(none)
553088	FITC Rat Anti-Mouse CD45R/B220	0.5 mg	RA3-6B2

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. Although hamster immunoglobulin isotypes have not been well defined, BD Biosciences Pharmingen has grouped Armenian and Syrian hamster IgG monoclonal antibodies according to their reactivity with a panel of mouse anti-hamster IgG mAbs. A table of the hamster IgG groups, Reactivity of Mouse Anti-Hamster Ig mAbs, may be viewed at http://www.bdbiosciences.com/pharmingen/hamster_chart_11x17.pdf.
4. 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

Pinckard JK, Sheehan KC, Arthur CD, Schreiber RD. Constitutive shedding of both p55 and p75 murine TNF receptors in vivo. *J Immunol.* 1997; 158(8):3869-3873.(Clone-specific)

Pinckard JK, Sheehan KC, Schreiber RD. Ligand-induced formation of p55 and p75 tumor necrosis factor receptor heterocomplexes on intact cells. *J Biol Chem.* 1997; 272(16):10784-10789.(Clone-specific: Immunoprecipitation)

Sheehan KC, Pinckard JK, Arthur CD, Dehner LP, Goeddel DV, Schreiber RD. Monoclonal antibodies specific for murine p55 and p75 tumor necrosis factor receptors: identification of a novel in vivo role for p75. *J Exp Med.* 1995; 181(2):607-617.(Clone-specific)

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.(Methodology: Flow cytometry)