PE Hamster Anti-Mouse CD120b

**Product Information**

- **Material Number:** 550086
- **Alternate Name:** Tnfrsf1b; Tnfr2; TNF-R2; TNFR p75; TNFRII; TNF-R-II; TNFBR
- **Size:** 0.2 mg
- **Concentration:** 0.2 mg/ml
- **Clone:** TR75-89
- **Immunogen:** Mouse Type II TNFR
- **Isotype:** Armenian Hamster IgG1, λ
- **Reactivity:** QC Testing: Mouse
- **Storage Buffer:** Aqueous buffered solution containing ≤0.09% sodium azide.

**Description**

The TR75-89 monoclonal antibody specifically binds to 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/10^6 cells] to block Fc receptor-mediated nonspecific staining by staining antibodies. The cells were stained (1 hr., 4°C) with R-PE conjugated TR75-89 antibody (1 µg mAb/10^6 cells; Cat. No. 550086). The cells were washed and were incubated with FITC- RA3-6B2/B220 (Cat. No. 553088; 0.06 µg mAb/10^6 cells), and were washed in preparation for flow cytometric analysis with a FACScan® Flow Cytometer. The immunofluorescent staining patterns for cells stained with either R-PE conjugated TR75-89 antibody (filled histogram) or streptavidin-PE (background staining; empty histogram) is 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., B20-negative lymph node T cells.

**Preparation and Storage**

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

**Application Notes**

**Application**

| Flow cytometry | Routinely Tested |

**Recommended Assay Procedure:**

For country contact information, visit [bdbiosciences.com/contact](http://bdbiosciences.com/contact)

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Immunofluorescent Staining and Flow Cytometric Analysis: The R-PE conjugated form of TR75-89 (Cat. No. 550086) can be used for the immunofluorescent staining (≤ 1 µg antibody/10^6 cells) and flow cytometric analysis of normal mouse cells or cell lines to measure their expressed levels of p75 TNFR. An appropriate R-PE-conjugated immunoglobulin isotype control is clone G235-2356 (Cat. No. 554711).

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 10^6 cells was insufficient to inhibit the binding of TR75-89 to L929 cells that express p75 TNFR (at 0.25 µg antibody/10^6 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 cells 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

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>554711</td>
<td>PE Hamster IgG1, λ1 Isotype Control</td>
<td>0.1 mg</td>
<td>G235-2356</td>
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<tr>
<td>553088</td>
<td>FITC Rat Anti-Mouse CD45R/B220</td>
<td>0.5 mg</td>
<td>RA3-6B2</td>
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<tr>
<td>553142</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
<td>0.5 mg</td>
<td>2.4G2</td>
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Product Notices
1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
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. 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/documents/hamster_chart_11x17.pdf.

References