Technical Data Sheet

Purified Rat Anti-Mouse CD210

Product Information

559912 **Material Number:**

Alternate Name: IL-10 Receptor

0.5 mg Size: **Concentration:** 0.5 mg/ml Clone: 1B1.3a

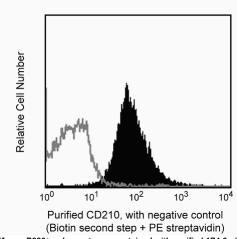
Purified recombinant ligand-binding domain of mIL-10R Immunogen:

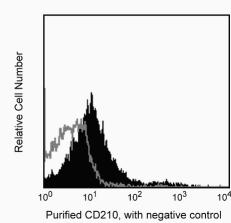
Rat IgG1, κ Isotype: Reactivity: QC Testing: Mouse

Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 1B1.3a antibody reacts with the extracellular region of mouse CD210 which is also known as the mouse IL-10 receptor (mIL-10R); it does not recognize the human IL-10R. The IL-10R is expressed by a variety of mouse cell types and cell lines including thymocytes, T cells, B cells, and monocytes. 1B1.3a is a neutralizing antibody and reportedly blocks the binding of human IL-10, which cross-reacts with the mouse IL-10R. The immunogen used for the generation of the 1B1.3a hybridoma was purified recombinant ligand-binding domain of mIL-10R.





(Biotin second step + PE streptavidin)

Mouse B220+ splenocytes were stained with purified 1B1.3a (0.25 μg, Cat. No. 559912) followed by biotinylated polyclonal goat anti-rat Ig (0.25 μg, Cat. No. 554014) and Streptavidin-PE (0.015 μg, Cat. No. 554061) in a three layer staining protocol to amplify immunofluorescent signals (left panel). In addition, cells in buffer containing sodium azide were pre-incubated on ice with recombinant mouse IL-10 (0.25 µg, Cat. No. 554585) prior to staining with 1B1.3a to determine the blocking effect of bound IL-10 protein on staining with 1B1.3a (right panel). Staining with the purified 1B1.3a antibody (filled histograms) is compared to the staining obtained with the secondary antibody alone (open histograms). Histograms in both panels are gated on the lymphocyte population as defined by light scattering characteristics and were also gated on the cell subset stained with FITC-conjugated rat-anti-mouse B220 (0.06 μg, Cat. No. 553088).

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

Recommended Assay Procedure:

Immunofluorescent staining and flow cytometric analysis: The R-PE-conjugated 1B1.3a antibody (Cat. No. 559912) can be used for immmunofluorescent staining ($\leq 1 \, \mu g$ antibody/10e6 cells) and flow cytometric analysis of the levels of membrane IL-10R expressed by mouse cell lines or mouse lymphoid cells. It is recommended that the purified format of this antibody can be used in conjunction with biotinylated polyclonal goat anti-rat Ig (Cat. No. 554014) and streptavidin-phycoerythrin (PE) (Cat. No. 554061) in a three-layer staining procedure to amplify immunofluorescent signals. An appropriate R-PE-conjugated immunoglobulin isotype-matched control is R3-34 (Cat. No. 554685).

Note: Since 1B1.3a is a neutralizing antibody, it competes with IL-10 for binding to its receptor. Therefore, the use of the 1B1.3a antibody for immunofluorescent staining and flow cytometric analysis in systems where the natural ligand of the receptor is present may give an underestimation of IL-10R expression. Based on our testing results, the presence of recombinant mouse IL-10 protein at levels above 250 ng/ml is sufficient to partially inhibit the binding of the 1B1.3a antibody (at 0.12 µg/10e6 cells).

In vitro neutralization: The NA/LE format of this antibody (Cat. No. 550012) is useful for bioassay.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554014	Biotin Goat Anti-Rat Ig	0.5 mg	Polyclonal
554061	PE Streptavidin	0.5 mg	(none)
553922	Purified Rat IgG1, κ Isotype Control	0.5 mg	R3-34
553088	FITC Rat Anti-Mouse CD45R/B220	0.5 mg	RA3-6B2
550012	Purified NA/LE Rat Anti-Mouse CD210	each	B27

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. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.

References

O'Farrell AM, Liu Y, Moore KW, Mui AL. IL-10 inhibits macrophage activation and proliferation by distinct signaling mechanisms: evidence for Stat3-dependent and -independent pathways. *EMBO J.* 1998; 17(4):1006-1018.(Immunogen: Neutralization)

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)

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