Technical Data Sheet

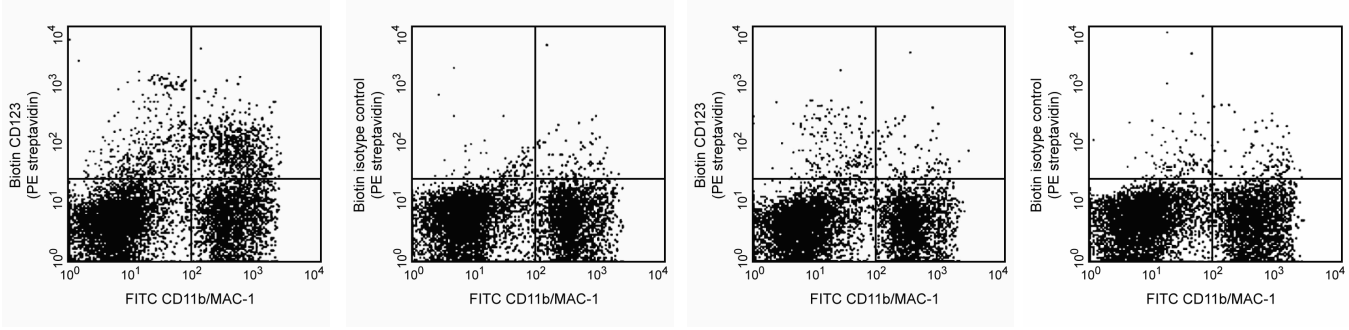
Biotin Rat Anti-Mouse CD123

Product Information

Material Number:	**555070**
Alternate Name:	IL-3 Receptor α chain
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	5B11
Immunogen:	Mouse IL-3R transfected CTLL cells
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The 5B11 antibody reacts with mouse CD123, the α chain subunit (IL-3Rα) of the mouse IL-3 receptor. The IL-3Rα chain is a 60-70 kD transmembrane glycoprotein that binds IL-3 with low affinity but cannot transduce signals by itself. The mouse IL-3Rα chain can complex with either of two homologous β chain subunits (βc and βIL-3) to form high-affinity heterodimeric IL-3 receptors. The βc chain is the common β chain subunit that can complex with the α subunits of the mouse IL-3R, IL-5R and GM-CSFR to form high-affinity receptors. The βIL-3 subunit can form high affinity IL-3 receptor complexes with only the IL-3Rα subunit. The βIL-3 subunit clone is specific for mouse IL-3 and binds with low affinity. The immunogen used to generate the 5B11 hybridoma was CTLL cells transfected with the mouse IL-3R alpha chain. The 5B11 antibody does not block the binding of IL-3 protein to the high affinity IL-3 receptor heterodimer.



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

Recommended Assay Procedure:

The biotinylated 5B11 antibody (Cat. No. 555070) can be used for the immunofluorescent staining and flow cytometric analyses of mouse leukocytes and cell lines that express IL-3 receptor α chains (see Figure). Using an indirect immunofluorescent staining technique (see below) the 5B11 antibody (0.25 µg/test) was found to positively stain a significantly higher proportion of bone marrow cells from C57B6 mice (First panel) as compared with cells from A/J mice (Third panel) as previously reported. The level of nonspecific staining was assessed using the biotin rat IgG2a isotype control (Cat. No. 553928; Second and final panel). Moreover, positive staining was also observed on the mouse IL-3-dependent cell lines, MC-9 and D36 (Data not shown).

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

A multistep step staining procedure is recommended to amplify immunofluorescent signals for the flow cytometric analysis of mouse IL-3Rα chain expression:

Step 1: Incubate the cells with 0.25 - 4 µg of biotinylated 5B11 antibody at 4°C for 15 - 20 minutes. Wash cells two times with staining medium containing sodium azide (e.g., Dulbecco's PBS or tissue culture medium [without phenol red and biotin] with 0.09% sodium azide and 2% heat-inactivated FCS or 0.2% BSA).
Step 2: Incubate the cells with . 0.5 µg of streptavidin-phycoerythrin (Cat. No. 554061) at 4°C for 20 minutes. Wash two times. Resuspend cells instaining medium and analyze stained cells with a FACScan. Flow Cytometer (Becton Dickenson, San Jose, CA) using appropriate specifity and compensation and compensation controls.

Suggested Companion Products

Catalog Number	Name	Size	Clone
553928	Biotin Rat IgG2a κ Isotype Control	0.25 mg	R35-95
554061	PE Streptavidin	0.5 mg	(none)

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.

References

Hara T, Miyajima A. Two distinct functional high affinity receptors for mouse interleukin-3 (IL-3). *EMBO J.* 1992; 11(5):1875-1884.(Biology)
Ichihara M, Hara T, Takagi M, Cho LC, Gorman DM, Miyajima A. Impaired interleukin-3 (IL-3) response of the A/J mouse is caused by a branch point deletion in the IL-3 receptor alpha subunit gene. *EMBO J.* 1995; 14(5):939-950.(Biology)
Mueller DL, Chen ZM, Schwartz RH, Gorman DM, Kennedy MK. Subset of CD4+ T cell clones expressing IL-3 receptor alpha-chains uses IL-3 as a cofactor in autocrine growth. *J Immunol.* 1994; 153(7):3014-3027.(Biology)