Technical Data Sheet

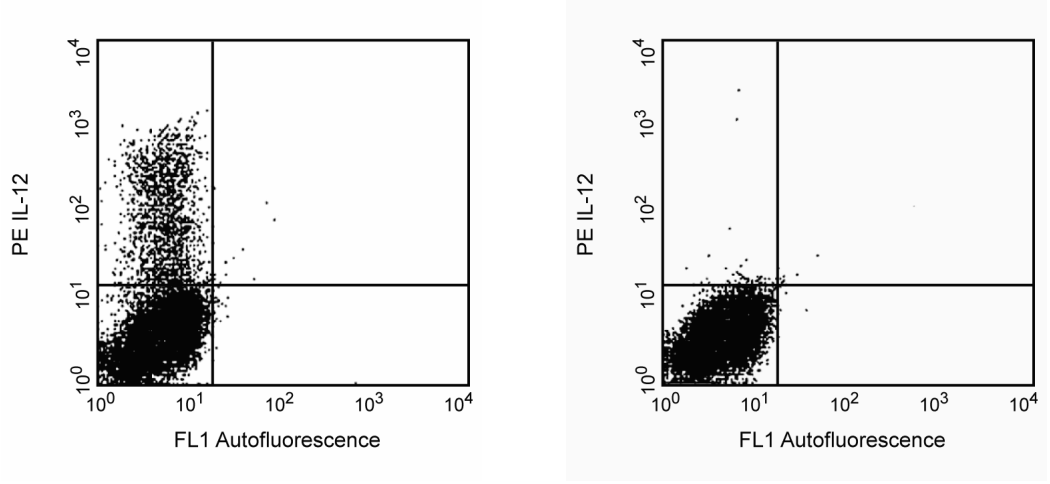
PE Rat Anti-Mouse IL-12 (p40/p70)

Product Information

Material Number:	**562038**
Alternate Name:	IL-12/IL-23 p40; Il12b; Interleukin 12b; CLMF p40
Size:	25 µg
Concentration:	0.2 mg/ml
Clone:	C15.6
Immunogen:	CHO-expressed recombinant mouse IL-12 p70 protein
Isotype:	Rat IgG1
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The C15.6 monoclonal antibody specifically binds to both free and complexed (homodimer p80 and heterodimer p70) forms of the p40 subunit of mouse interleukin-12 (IL-12). The immunogen used to generate the C15.6 hybridoma was recombinant mouse IL-12 p70 protein. p40 has also been described as a subunit of IL-23 and thus it is possible that the C15.6 antibody will crossreact with IL-23.



Expression of IL-12 by mouse bone marrow-derived macrophages. *Bone marrow cells from 6 month old BALB/c mice were cultured for 10 days in mouse GM-CSF (40 ng/ml final concentration; Cat. No. 554586). Adherent cells were washed and treated for ~14 hours with mouse IFN-γ (10 ng/ml final concentration; Cat. No. 554587); subsequently LPS (1 µg/ml final concentration; Sigma) and GolgiStop™ (2 µM final concentration; Cat. No. 554724) were added to cultures for an additional 5 hours. Adherent cells were washed and then incubated with 1x trypsin EDTA at 37°C for 15 minutes and gently dislodged by pipetting. Nonspecific surface binding was blocked by incubation of cells with purified polyclonal normal mouse immunoglobulin. Cells were then fixed, permeabilized, and non-specific binding to intracellular antigens was blocked using BD Cytofix/Cytoperm™ (Cat. No. 554714). Cells were then stained with 0.06 µg of PE Rat anti-Mouse IL-12 (p40/p70) antibody (PE-C15.6, Cat. No. 562038; see left panel) using Pharmingen's staining protocol. To demonstrate specificity of staining, the binding of PE-C15.6 antibody was blocked by the preincubation of the fixed/permeabilized cells with unlabeled C15.6 antibody (5 µg/ml final concentration; Cat. No. 554477; see right panel) prior to staining. The quadrant markers for the bivariate dot plots were set based on the autofluorescence control and verified using the ligand blocking and unlabeled antibody blocking controls.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

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

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The C15.6 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-12 producing cells within mixed cell populations. The PE- and APC-conjugated C15.6 antibodies are especially suitable for these experiments (see figure). For optimal immunofluorescent staining and flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, see The Immune Function Handbook at our website at www.bdbiosciences.com.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the PE-C15.6 antibody with excess ligand prior to staining, or 2) pre-block paraformaldehyde-fixed/saponin-permeabilized cells with unlabeled C15.6 antibody (e.g., Cat. No. 554477) prior to staining. The intracellular staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable rat IgG1 isotype control immunoglobulin for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized mouse or human cells is PE-R3-34 immunoglobulin (Cat. No. 554685); use at comparable concentrations to antibody of interest.

ELISA: The purified C15.6 antibody (Cat. No. 551219) is useful as a capture antibody for a sandwich ELISA for measuring mouse IL-12 p40 protein levels. The purified C15.6 antibody can be paired with the biotinylated C17.8 antibody (Cat. No. 554476) as the detection antibody, with recombinant mouse IL-12 p40 protein (Cat. No. 554594) as the standard. For testing mouse IL-12 p40 in complex biological fluids such as serum or plasma, our mouse IL-12 specific OptEIA™ sandwich ELISA set is recommended (Cat. No. 555165).

Suggested Companion Products

Catalog Number	Name	Size	Clone
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
554654	MiCK-3 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
554685	PE Rat IgG1, κ Isotype Control	0.1 mg	R3-34

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. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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.
5. An isotype control should be used at the same concentration as the antibody of interest.

References

Prussin C, Metcalfe DD. Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. *J Immunol Methods.* 1995; 188(1):117-128. (Methodology: IC/FCM Block)

Wysocka M, Kubin M, Vieira LQ, et al. Interleukin-12 is required for interferon-gamma production and lethality in lipopolysaccharide-induced shock in mice. *Eur J Immunol.* 1995; 25(3):672-676. (Clone-specific: ELISA)