Technical Data Sheet

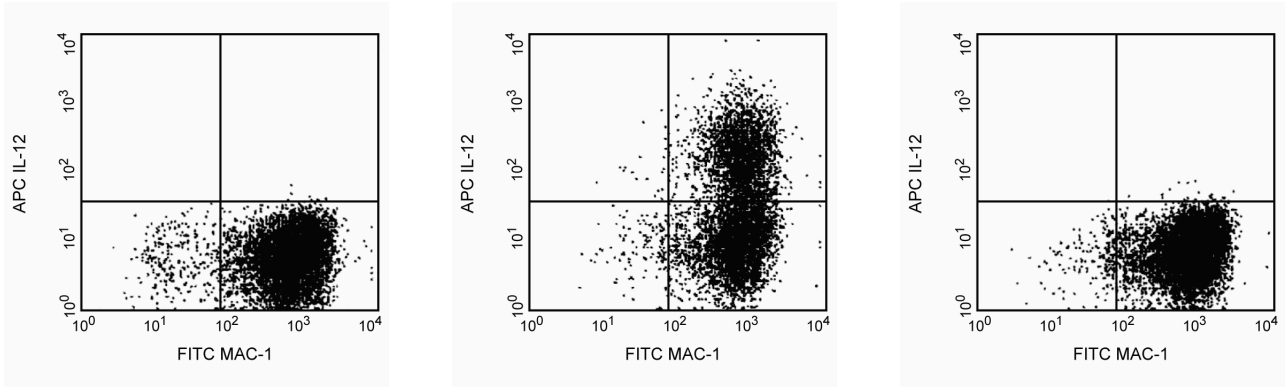
APC Rat Anti-Mouse IL-12 (p40/p70)

Product Information

Material Number:	**554480**
Size:	0.1 mg
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, Cat. No. 554586). Adherent cells were washed and treated for ~14 hr with mouse IFN-γ (10 ng/ml, 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 hr. 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 surface stained with 0.06 µg of FITC-conjugated rat anti-mouse CD11b (MAC-1) antibody (Cat. No. 553310; see left panel). Cells were then fixed, permeabilized, and nonspecific binding to intracellular antigens was blocked using PBS/2%BSA/0.1% saponin. Cells were then stained with 0.25 µg of APC-conjugated rat anti-mouse IL-12 (p40/p70) antibody (APC-C15.6, Cat. No. 554480; see middle panel) using the staining protocol. To demonstrate specificity of staining, the binding of APC-C15.6 antibody was blocked by the preincubation of cells with unlabeled C15.6 antibody (5.0 µg/ml, Cat. No. 554477; see right panel) prior to staining with APC-C15.6. 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. This APC-conjugated reagent can be used in any flow cytometer equipped with a a dye, HeNE or red diode laser. These include the dual laser FACStarPLUS™, FACS Vantage™ or FACSCalibur™.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to APC under optimum conditions, and unconjugated antibody and free APC were removed.

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

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Immunofluorescent Staining and Flow Cytometric Analysis: The APC-conjugated C15.6 antibody can be used for multicolor immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-12 p40/p70-producing cells within mixed cell populations (see figure). For optimal immunofluorescent staining with flow cytometric analysis, this anti-cytokine antibody should be titrated (≤ 0.5 µg mAb/million cells). For specific methodology, please see the chapter on intracellular staining and flow cytometry in the Immune Function Handbook, or the visit the online protocols, both of which are found on our web site, www.bdbiosciences.com.

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A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the APC-C15.6 antibody with excess ligand (e.g., recombinant mouse IL-12 p40 protein, Cat. No. 554594) 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 APC-R3-34 immunoglobulin (Cat. No. 554686); 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 (Cat. No. 554594) as the standard. For specific methodology, please visit the protocols online or the chapter on ELISA in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com. For testing mouse IL-12 p40 in complex biological fluids like 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)
554686	APC Rat IgG1, κ Isotype Control	0.1 mg	R3-34
554654	MiCK-3 Mouse Cytokine Positive Control Cells	1.0 ml	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(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. 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.

References

Gately MK, Chizzonite R, Presky DH. Measurement of Human and Mouse Interleukin-12. In: Cooligan J, Kruisbeek A, Margulies D, Shevach E, Storber W, ed. *Current Protocols in Immunology.* New York: John Wiley and Sons; 1995:6-16. (Clone-specific: ELISA)

Oppmann B, Lesley R, Blom B, et al. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity.* 2000; 13(5):715-725. (Biology)

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)