Technical Data Sheet Purified Rat Anti-Mouse IL-12 (p40/p70)

Product Information	
Material Number:	554477
Size:	0.1 mg
Concentration:	0.5 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 antibody reacts with 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.

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 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 PBS/2%BSA/0.1% saponin. Cells were then stained with 0.06 µg of PE-conjugated rat anti-mouse IL-12 (p40/p70) antibody (PE-C15.6, Cat. No. 55479; see left panel) using the BD Pharmingen[™] staining protocol. To demonstrate specificity of staining, the binding of PE-C15.6, antibody was blocked by the preincubation of the conjugated antibody with recombinant mouse IL-12 p40 protein (0.5 µg, Cat. No. 554594; see middle panel), and by preincubating 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 Iigand blocking and unlabeled antibody blocking controls.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. Store undiluted at 4° C.

Application Notes

Applicatio	n						
Intracellular block/flow cytometry					F	Routinely Tested	
BD Bioscier	nces						
bdbiosciences	.com						
United States	Canada	Europe	Japan	Asia Pacific	Latin America/Ca	aribbean	MAD
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Recommended Assay Procedure:

Blocking Control for Intracellular Staining: The purified C15.6 antibody (Cat. No. 554477) can be used as a blocking control to demonstrate specificity of IL-12 staining by conjugated C15.6 antibody (Cat. No. 554479 and 554480). To perform this control, the fixed/permeabilized cells (~1 million) can be incubated with 1 -10 µg of unlabeled C15.6 antibody (Cat. No. 554477) for 20 minutes at 4°C, prior to staining with PE-conjugated C15.6 antibody (e.g., 0.1 - 0.5 µg mAb/1 million cells). The intracellular cytokine staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. For specific methodology, please visit our web site, www.bdbiosciences.com, and go to the protocols section or the chapter on intracellular staining in the Immune Function Handbook.

ELISA Capture- IL-12 p40 ELISA: The purified C15.6 antibody is useful as a capture antibody for a sandwich ELISA measuring the total amount of p40 in solution (complexed as homodimer or heterodimer, as well as free monomer). The purified C15.6 antibody, Cat. No. 551219 is recommended for use in ELISA applications. C15.6 antibody can be paired with the biotinylated C17.8 antibody (Cat. No. 554476) as the detecting antibody, with recombinant mouse IL-12 p70 protein (Cat. No. 554592), or IL-12 p40 protein (Cat. No. 554594) as the standard. Note: For testing mouse IL-12 p40 in complex biological fluids like serum or plasma, our mouse IL-12p40 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)	
554594	Recombinant Mouse IL-12 (p40)	2 µg	(none)	
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)	
554654	Mick-3 Cytokine Positive Control Cells	25 mg	(none)	
554479	PE Rat Anti-Mouse IL-12 (p40/p70)	0.1 mg	C15.6	
554480	APC Rat Anti-Mouse IL-12 (p40/p70)	0.1 mg	C15.6	

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

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)