Technical Data Sheet

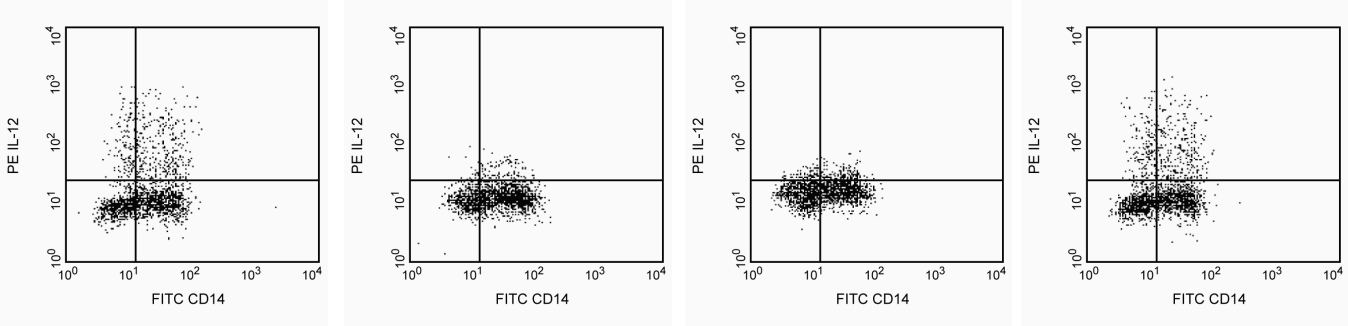
PE Mouse Anti-Human IL-12 (p40/p70)

Product Information

Material Number:	**554575**
Size:	0.1 mg
Concentration:	0.2 mg/ml
Clone:	C11.5
Immunogen:	CHO-expressed recombinant human IL-12 p70 heterodimer
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The C11.5 monoclonal antibody specifically binds to the human IL-12 p40 monomer and p70 heterodimer, but does not bind to the IL-12 p35 monomer. The immunogen used to generate the C11.5 hybridoma was the CHO-expressed recombinant human IL-12 p70 heterodimer. p40 has also been described as a subunit of IL-23 and thus it is possible that the C11.5 antibody crossreacts with IL-23.



Expression of IL-12 p40/p70 by activated CD14+ human PBMCs. *Ficoll-separated human PBMCs were primed for 2 hours with recombinant human IFN-γ (10 ng/ml final concentration; then activated with IFN-γ (10 ng/ml final concentration) and LPS (100 ng/ml final concentration; Sigma) in the presence of GolgiStop™ (2 µM final concentration) for an additional 22 hours. Cells were harvested, stained with FITC-mouse anti human CD14 antibody fixed, permeabilized, and then stained with 0.125 µg of PE-C11.5 antibody following our staining protocol (far left panel). The data reflect gating on monocytes, based on forward and side scattered light signals. To demonstrate specificity of staining, the binding of PE-C11.5 antibody was blocked by preincubation of the conjugated antibody with excess recombinant human IL-12 p70 (0.25 µg) and recombinant human IL-12 p40, but not by recombinant human IL-12 p35 protein. Preincubation of the fixed/permeabilized cells with an excess of the unlabelled C11.5 antibody prior to staining with the PE-C11.5 antibody also blocked staining (data not shown). The quadrant markers for the bivariate dot plots were set based on the autofluorescence control, and verified with the recombinant cytokine blocking and unlabelled antibody blocking specificity controls.*

Preparation and Storage

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.
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 C11.5 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-12 producing cells within mixed cell populations. The FITC-, PE-, or APC-conjugated C11.5 antibodies are especially suitable for these studies (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 visit the protocols section or chapter on intracellular staining in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

A useful control for demonstrating specificity of staining is either of the following: 1) pre-block the fluorochrome-conjugated C11.5 antibody with excess ligand (e.g., recombinant human IL-12 p70, or recombinant human IL-12 p40, prior to staining, or 2) pre-block the fixed/permeabilized cells with unlabelled C11.5 antibody prior to staining. The intracellular staining technique and use of blocking controls are described in detail by C. Prussin and D. Metcalfe. A suitable mouse IgG1 isotype control for assessing the level of background staining on paraformaldehyde-fixed/saponin-permeabilized human cells is PE-MOPC-21 clone (Cat. No. 554680); use at comparable concentrations to antibody of interest (e.g., ≤ 0.5 µg mAb/1 million cells).

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Suggested Companion Products

Catalog Number	Name	Size	Clone
554680	PE Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
555063	HiCK-3 Human Cytokine Positive Control Cells	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.
5. Ficoll-Paque is a trademark of Amersham Biosciences Limited.

References

D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med.* 1993; 178(3):1041-1048. (Clone-specific)

D'Andrea A, Rengaraju M, Valiante NM, et al. Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. *J Exp Med.* 1992; 176(5):1387-1398. (Clone-specific)

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)

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)