Technical Data Sheet

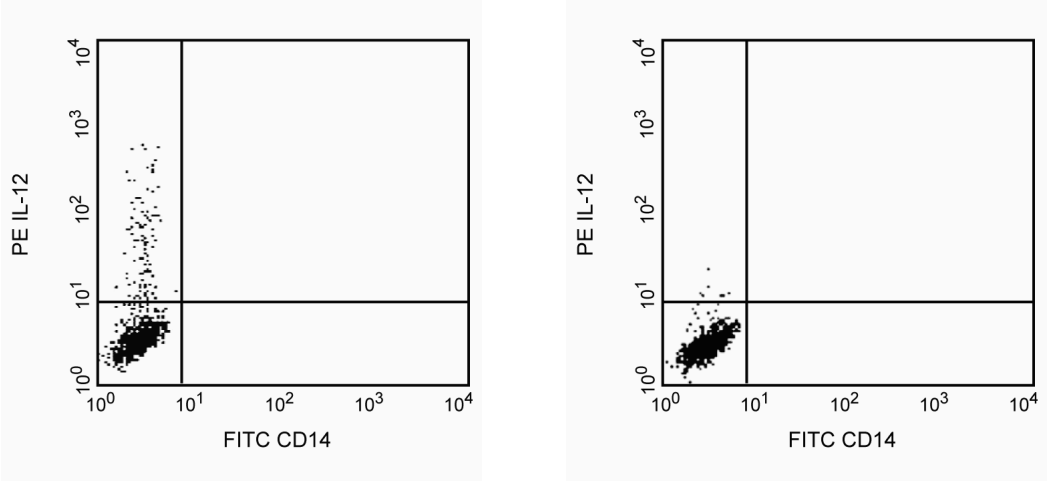
PE Mouse Anti-Human IL-12 (p40/p70)

Product Information

Material Number:	**559329**
Size:	100 tests
Vol. per Test:	20 µl
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 BSA and ≤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 HiCK-3 cells. *Ficoll™-separated human PBMCs were primed for 2 hours with recombinant human IFN-γ (20 ng/ml final concentration; Cat. No. 554616), then activated with IFN-γ(20 ng/ml final concentration) and LPS (1 µg/ml final concentration; Sigma) in the presence of BD GolgiStop™ (2 µM final concentration; Cat. No. 554724) for an additional 22 hours. Cells were harvested, fixed, permeabilized, and then stained with 20 µl of PE-C11.5 antibody (Cat. No. 559329), by following the Usage section above and Pharmingen's staining protocol (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 an excess of the unlabelled C11.5 antibody (5 µg, Cat. No. 554573; right panel). 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. This 100 Test Size formulation of the PE-conjugated C11.5 antibody has been pre-titrated to assure effective intracellular detection of human IL-12 using 20 µl/1 x 10e6 cells.

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, (Cat. No. 554613) or recombinant human IL-12 p40, (Cat. No. 554633) prior to staining, or 2)

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pre-block the fixed/permeabilized cells with unlabelled C11.5 antibody (Cat. No. 554573) 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 also available in a 100 Test Size formulation PE-MOPC-21 (Cat. No. 559320).

Important Note: This pre-titered antibody solution does not contain a cell permeabilization agent. It is necessary to include a cell permeabilization agent when using the pre-titered antibody solution to stain fixed and permeabilized cells. BD Perm/Wash™ Buffer (Cat. No. 554723) contains the permeabilization agent saponin and is useful for this purpose as described in the Usage section.

Usage

1. Resuspend 1 x 10e6 fixed and permeabilized cells in 20 µl of the pre-titered antibody solution and 30 µl of 1X BD Perm/Wash Buffer (Cat. No. 554723).
2. Incubate the cell suspension for 15 minutes (at RT or 4°C).
3. Wash twice in 100 µl of 1X BD Perm/Wash Buffer (Cat. No. 554723).

Suggested Companion Products

Catalog Number	Name	Size	Clone
554573	Purified Mouse Anti-Human IL-12 (p40/p70)	0.1 mg	C11.5
554723	Perm/Wash Buffer	100 ml	(none)
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
554613	Recombinant Human IL-12 (p70)	5 µg	(none)
554633	Recombinant Human IL-12 p40	5 µg	(none)
555063	HiCK-3 Human Cytokine Positive Control Cells	1.0 ml	(none)
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. 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: IC/FCM Block)