Technical Data Sheet

V450 Mouse Anti-Human IL-12 (p40/p70)

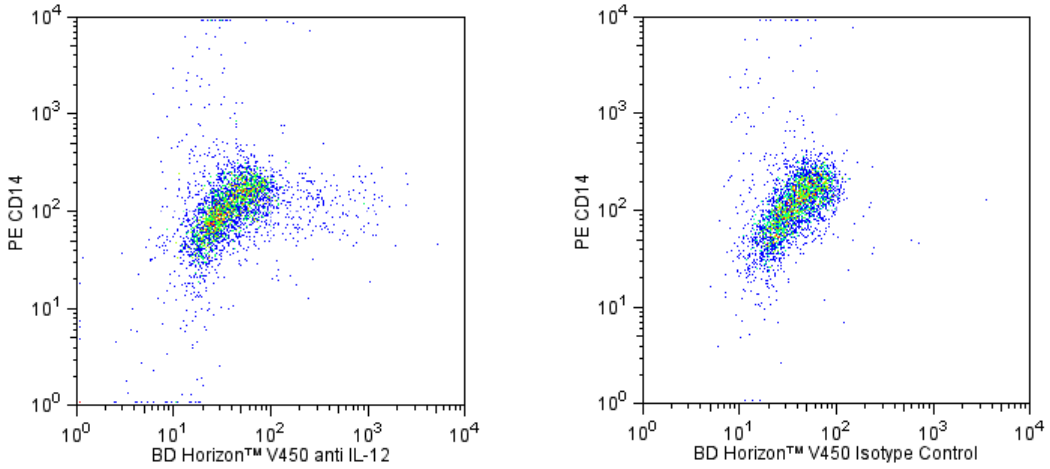
Product Information

Material Number:	**561380**
Alternate Name:	IL12B; IL-12B; CLMF; CLMF2; NKSF; NKSF2
Size:	50 tests
Vol. per Test:	5 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 protein stabilizer 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.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser Ex max of 406 nm and has an Em Max at 450 nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



Expression of IL-12 (p40/p70) by stimulated human monocytes. *Human peripheral blood mononuclear cells were primed for 2 hours with recombinant human IFN-γ (Cat. No. 554616/554617; 10 ng/ml final concentration). The cells were then activated with IFN-γ (10 ng/ml final concentration) and LPS (100 ng/ml final concentration) in the presence of GolgiStop™ (Cat. No. 554724) for an additional 22 hours. The cells were fixed using BD Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized with BD Perm/Wash™ Buffer (Cat. No. 554723). The cells were then stained with PE Mouse Anti-Human CD14 (Cat No. 555398) and BD Horizon™ V450 Mouse Anti-Human IL-12 (p40/p70) (Cat No. 561380, Left Panel) or BD Horizon™ V450 Mouse IgG1 κ Isotype Control (Cat No. 560373, Right Panel) by using BD Biosciences Intracellular Cytokine Staining protocol. Two-color flow cytometric dot plots showing the correlated expression of IL-12 (p40/p70) (or Ig Isotype Control Staining) versus CD14 were derived from events with the forward and side light-scatter characteristics of intact monocyte/macrophages. HiCK-3 Human Cytokine Positive Control cells (Cat No. 555063) are prepared in a similar manner. These cells can be used as a positive control for cytokine flow cytometry experiments designed to characterize the nature of human IL-12 (p40/p70)-producing cells. Flow cytometry was performed using a BD™ LSR II Flow Cytometer System.*

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Preparation and Storage

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

The antibody was conjugated with BD Horizon™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was 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

Suggested Companion Products

Catalog Number	Name	Size	Clone
554617	Recombinant Human IFN-γ	50 µg	(none)
554616	Recombinant Human IFN-γ	25 µg	(none)
554724	Protein Transport Inhibitor (Containing Monensin)	0.7 ml	(none)
554655	Fixation Buffer	100 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)
560373	V450 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
555398	PE Mouse Anti-Human CD14	100 tests	M5E2
555063	HiCK-3 Human Cytokine Positive Control Cells	1.0 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
4. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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)