Technical Data Sheet

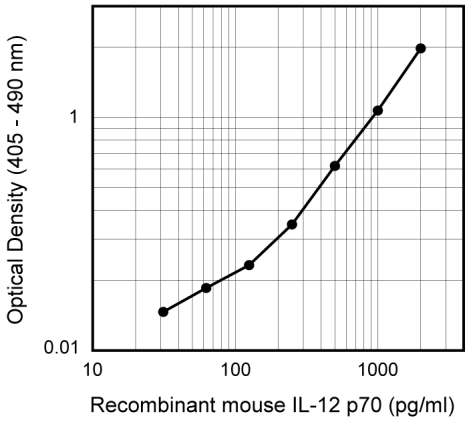
Biotin Rat Anti-Mouse IL-12 (p40/p70)

Product Information

Material Number:	**554476**
Size:	0.5 mg
Concentration:	0.5 mg/ml
Clone:	C17.8
Immunogen:	Recombinant Mouse IL-12 p70
Isotype:	Rat IgG2a
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The C17.8 antibody reacts with the free p40 monomer of IL-12 as well as the p40 subunit complexed as homodimer or complexed with p35 as a p70 heterodimer. Clone C17.8 has also been shown to be cross-reactive with mouse IL-23, which also contains the p40 subunit. The immunogen used to generate C17.8 hybridoma was recombinant mouse IL-12 p70.



ELISA Standard curve using recombinant mouse IL-12 p70 protein standard.

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with biotin under optimum conditions, and unreacted biotin was removed.

Application Notes

Application

ELISA Detection	Routinely Tested
Immunoprecipitation	Reported
Western blot	Reported

Recommended Assay Procedure:
The C17.8 clone is useful in a sandwich ELISA for mouse IL-12 (p40) and for mouse IL-12 (p70). For specific methodology please visit the protocols sections or the chapter on ELISA in the Immune Function Handbook, both of which are posted on our web site, www.bdbiosciences.com.

ELISA Detection (IL-12 p70 ELISA): The biotinylated C17.8 antibody (Cat. No. 554476) is useful as a detection antibody for a sandwich ELISA for specifically measuring mouse IL-12 p70 protein levels. Biotinylated C17.8 antibody can be paired with anti-mouse IL-12 (p70) specific antibody 9A5 (Cat. No. 554658), as the capture antibody with recombinant mouse IL-12 p70 (Cat. No. 554592) as the standard. The biotinylated C17.8 antibody should be titrated 0.5 - 2.0 µg/ml to determine optimal concentration for ELISA detection. To obtain linear standard curves, doubling dilutions of mouse IL-12 protein ranging from ~2000 to 15 pg/ml are recommended for inclusion in each ELISA plate. For maximal sensitivity, an overnight incubation (4°C) of samples/standards with the coated capture antibody is recommended.

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Important Note: This mouse IL-12 p70 ELISA (Cat. No. 554658/Cat. No. 554476) for measuring mouse IL-12 p70 protein levels is not interfered with by excess p40 monomer. This assay is specific for measuring mouse IL-12 p70 protein. This is advantageous because it has been reported that cells producing IL-12 p70 protein can coexpress high levels of p40 monomer.

ELISA Detection (IL-12 p40 ELISA): The biotinylated C17.8 antibody is useful as a detection antibody for a sandwich ELISA measuring IL-12 p40 protein levels (free p40 monomer as well as p40 complexed as homodimer or heterodimer). Biotinylated C17.8 antibody can be paired with the purified C15.6 antibody (Cat. No. 551219) as the capture antibody, and with recombinant mouse IL-12 p70 protein (Cat. No. 554592; see figure) or IL-12 p40 protein (Cat. No. 554594; data not shown) as the standard. The biotinylated C17.8 antibody should be titrated 0.5 - 2.0 µg/ml to determine optimal concentration for ELISA detection. To obtain linear standard curves, doubling dilutions of mouse IL-12 protein ranging from ~2000 to 15 pg/ml are recommended for inclusion in each ELISA plate. For maximal sensitivity, an overnight incubation (4°C) of samples/standards with the coated capture antibody is recommended.

Neutralization/Blocking: The NA/LE format of the C17.8 antibody (Cat. No. 554475) is useful for neutralization of mouse IL-12 p70 bioactivity. The NA/LE preparation is tested in bioassay to verify neutralizing activity.

Western Blot/IP: The C17.8 antibody has been reported to be useful for immunoprecipitation and Western blotting studies. Please note that this application is not routinely tested at BD Biosciences.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554658	Purified Rat Anti-Mouse IL-12 p70	0.5 mg	9A5
554592	Recombinant Mouse IL-12 p70	5 µg	(none)
554594	Recombinant Mouse IL-12 (p40)	2 µg	(none)
554475	Purified NA/LE Rat Anti-Mouse IL-12 (p40/p70)	0.5 mg	C17.8
551219	Purified Rat Anti-Mouse IL-12 p40/p70	1.0 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

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. (Biology)

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. (Biology)

Neurath MF, Fuss I, Kelsall BL, Stuber E, Strober W. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J Exp Med.* 1995; 182(5):1281-1290. (Clone-specific: Neutralization)

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: Neutralization)

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