

## Technical Data Sheet

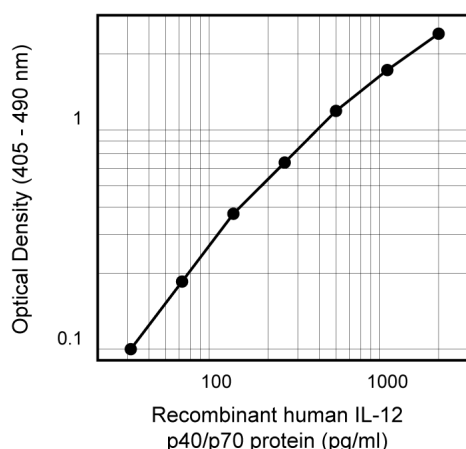
**Biotin Mouse Anti-Human IL-12 (p40/p70)****Product Information**

<b>Material Number:</b>	<b>554660</b>
<b>Size:</b>	0.5 mg
<b>Concentration:</b>	0.5 mg/ml
<b>Clone:</b>	C8.6
<b>Immunogen:</b>	Purified p40 Subunit of Human IL-12
<b>Isotype:</b>	Mouse IgG1
<b>Reactivity:</b>	QC Testing: Human
<b>Storage Buffer:</b>	Aqueous buffered solution containing ≤0.09% sodium azide.

**Description**

The C8.6 antibody reacts with human IL-12 p40 monomers, homodimers, and p70 heterodimers, but not p35 subunits. The immunogen used to generate the C8.6 hybridoma was the purified p40 subunit of human IL-12. The C8.6 antibody is a neutralizing antibody and has been reported to strongly inhibit three different biological activities of IL-12 (i.e., IFN- $\gamma$  induction), mitogenic effects on PHA blasts, and enhancement of NK cell-mediated cytotoxicity. C8.6 has been shown to immunoprecipitate IL-23.

This antibody is routinely tested by ELISA as a detection antibody. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



**Human IL-12 p70 Protein Quantitation by ELISA.** Rat anti-human IL-12 p70 specific antibody 20C2 (Cat. No. 555065) was paired with a biotinylated anti-IL-12 p40/p70 detection (Cat. No. 554660) to measure specifically human IL-12 p70 protein, using recombinant human IL-12 p70 (Cat. No. 554613), as the ELISA standard.

**Preparation and Storage**

The antibody was conjugated with biotin under optimum conditions, and unreacted biotin was removed.

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

Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

**Application Notes****Application**

ELISA Detection

Routinely Tested

**Recommended Assay Procedure:**

**ELISA Detection (IL-12 p70 protein ELISA):** The biotinylated C8.6 antibody (Cat. No. 554660) is useful as a detection antibody for a sandwich ELISA for specifically measuring human IL-12 p70 protein levels. Biotinylated C8.6 antibody can be paired with the purified rat anti-human IL-12 p70 specific 20C2 (Cat. No. 555065) as the capture antibody and recombinant human IL-12 p70 protein (Cat. No. 554613) as the standard. Biotinylated C8.6 antibody should be titrated (0.5 - 2.0  $\mu$ g/ml) to determine optimal concentration for ELISA detection. To obtain linear standard curves, doubling dilutions of human IL-12 p70 ranging from ~2000 to 15 pg/ml are recommended for inclusion in each ELISA plate. For testing IL-12 p70 in serum or plasma BD OptEIA™ Set (Cat. No. 555183) or BD OptEIA™ Kit (Cat. No. 559258) is recommended.

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**Note 1:** This ELISA for measuring human IL-12 p70 protein levels is not interfered with by a 50-fold excess (by mass) of the human p40 monomer. This assay is specific for measuring human IL-12 p70 protein. It has been reported that activated PBMC produce an excess of free p40 protein over the p70 heterodimer.

**ELISA Detection (IL-12 p40 protein ELISA):** The biotinylated C8.6 antibody (Cat. No. 554660) is useful as a detection antibody for specifically measuring human IL-12 p40 protein levels, with no reactivity to human IL-12 p70 protein. Biotinylated C8.6 antibody can be paired with the purified (exclusively p40 specific) C8.3 antibody (Cat. No. 551227) as the capture antibody with recombinant human IL-12 p40 (Cat. No. 554633) as the standard. For testing IL-12 (p40) in complex biological fluids like serum or plasma BD OptEIA™ Set (Cat. No. 555171) or BD OptEIA™ Kit (Cat. No. 551116) is recommended.

**Note 2:** These ELISA pairs show no cross-reactivity with any of the cytokines or chemokines tested (e.g., human IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-13, IL-15, G-CSF, GM-CSF, IFN- $\gamma$ , lymphotactin, MCP-1, MCP-2, MIP-1 $\alpha$ , MIP-1 $\beta$ , NT-3, PDGF-AA, sCD23, SCF, TNF, Lt- $\alpha$ , VEGF; mouse IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 p70, IL-15, GM-CSF, IFN- $\gamma$ , MCP-1, TCA-3, TNF; rat IL-2, IL-4, IL-6, IL-10, GM-CSF, IFN- $\gamma$ , TNF).

**Neutralization:** The C8.6 antibody has been reported to be useful for neutralization of human IL-12 p70 bioactivity. A formulation that does not contain sodium azide formulation (NA/LE™) is available (Cat. No. 554659) and is recommended for functional assay. This application is not routinely tested at BD Biosciences Pharmingen.

### Suggested Companion Products

Catalog Number	Name	Size	Clone
555183	BD OptEIA™ Human IL-12 (p70) ELISA Set	20 tests	(none)
554633	Recombinant Human IL-12 p40	5 $\mu$ g	(none)
559258	BD OptEIA™ Human IL-12 (p70) ELISA Kit	2 plates	(none)
551116	BD OptEIA™ Human IL-12 (p40) ELISA Kit II	2 plates	(none)
554613	Recombinant Human IL-12	5 $\mu$ g	(none)
555171	BD OptEIA™ Human IL-12 (p40) ELISA Set	each	(none)
551227	Purified Mouse Anti-Human IL-12 p40	1.0 mg	C8.3
555065	Purified Rat Anti-Human IL-12 p70	0.5 mg	20C2

### Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to [wwwbdbiosciences.com/pharmingen/protocols](http://wwwbdbiosciences.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.(Clone-specific: Neutralization)

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)

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.(Clone-specific: Immunoprecipitation)