



Monoclonal Anti-human IL-12/IL-23 p40 Antibody

ORDERING INFORMATION

Catalog Number: MAB1510

Clone: 24901

Lot Number: CIK01

Size: 500 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human IL-12/IL-23 p40

Immunogen: Sf 21-derived rhIL-12 p40/p35 heterodimer

Ig class: mouse IgG₁

Applications: Neutralization of bioactivity
ELISA

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, Sf 21-derived, recombinant human interleukin 12 (rhIL-12). The IgG fraction of the ascites fluid was purified by Protein G affinity chromatography. p40 is a common subunit that occurs in the heterodimeric cytokines, IL-12 and IL-23.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level

< 0.1 EU per 1 µg of the antibody as determined by the LAL method.

Reconstitution

Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a **manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody recognizes human IL-12 (p40/p35 heterodimer), human IL-23 (p40/p19 heterodimer) and p40 homodimers. In direct ELISAs, this antibody shows no cross-reactivity with rmlIL-12 p40 or rplIL-12.

Neutralization of Human IL-23 Bioactivity

The exact concentration of antibody required to neutralize human IL-23 activity is dependent on the cytokine concentration, cell type, growth conditions and the type of activity studied. To provide a guideline, R&D Systems has determined the neutralization dose for this antibody under a specific set of conditions. The **Neutralization Dose₅₀ (ND₅₀)** for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response. The ND₅₀ for this lot of anti-human p40 antibody was determined to be approximately 0.02 - 0.1 µg/mL in the presence of 0.75 ng/mL of human IL-23, using IL-17 production by mouse splenocytes as an assay. The specific conditions are described in the figure legends.

This antibody also neutralizes hIL-12 induced activity of human PBLs. The ND₅₀ in this assay is approximately 0.05 - 0.25 µg/mL in the presence of 1 ng/mL rhIL-12.

Additional Application

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human IL-12/23 p40. The detection limit for rhIL-12 is approximately 2 ng/well.

Optimal dilutions should be determined by each laboratory for each application

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R&D Systems, Inc.
1-800-343-7475

Figure 1

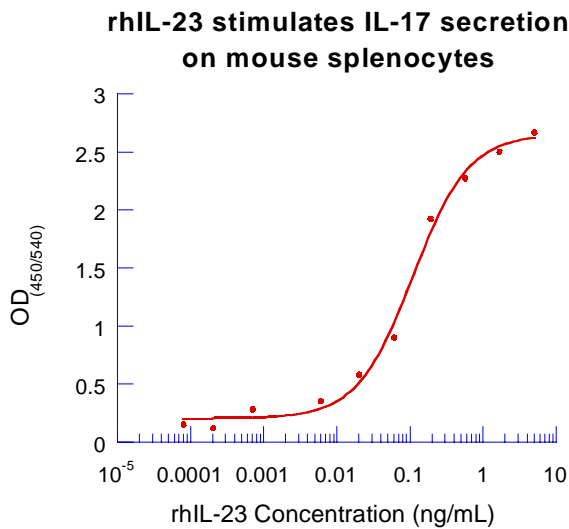


Figure 2

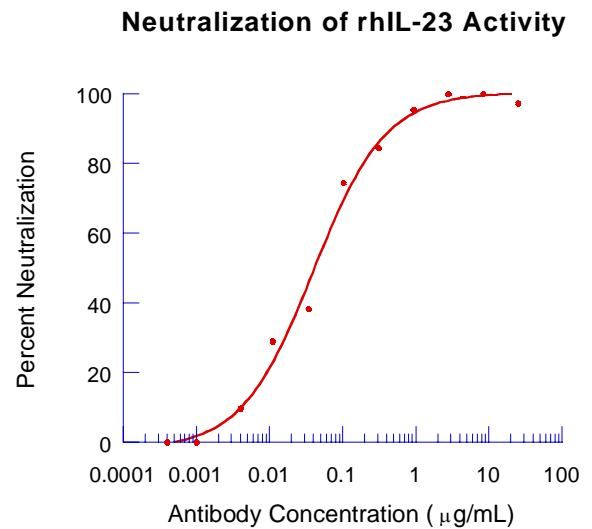


Figure 1

Human IL-23 stimulates the secretion of IL-17 on mouse splenocytes in a dose-dependent manner (Aggarwal, S. *et al.*, 2003, J. Biol. Chem. **278**:1910 - 1914). The ED₅₀ for this effect is typically 0.05 - 0.3 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the bioactivity of rhIL-23 on mouse splenocytes, rhIL-23 was incubated with various concentrations of antibody for 1 hour at 37° C in a 96 well microtiter plate. Following this preincubation period, mouse splenocytes were added. The assay mixture, in a total volume of 100 μL, containing antibody at the concentrations indicated, rhIL-23 at 0.75 ng/mL, rIL-2 at 10 ng/mL and cells at 2 x 10⁶ cells/mL, was incubated at 37° C for 3 days in a humidified CO₂ incubator. At the end of incubation, the amount of IL-17 secretion was measured by mouse IL-17 Quantikine kit (R&D Systems, Catalog # M1700). The ND₅₀ of the antibody is approximately 0.02 - 0.1 μg/mL.