Anti-human MCP-3 Neutralizing Antibody

ORDERING INFORMATION

Catalog Number: AB-282-NA

Lot Number: RF04

Size: 1 mg

Formulation: sterile solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rhMCP-3

Antigen: *E. coli*-derived rhMCP-3

Ig class: goat IgG

Applications: Neutralization of bioactivity
Western blot
ELISA

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant human monocyte chemotactic protein 3 (rhMCP-3). Total IgG was purified by Protein G affinity chromatography.

Formulation

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

Endotoxin Level

< 10 ng per 1 mg 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 1 mg/mL.

Storage

Lyophilized samples are stable for greater than 6 months at -20° C to -70° C. Reconstituted antibody is stable for at least 1 month at 2° - 4° C or 3 months at -20° C to -70° C under sterile conditions. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody has been selected for its ability to neutralize the biological activity of rhMCP-3. Based on direct ELISA and western blot results, this antibody shows less than 6% cross-reactivity with rhMCP-2 and rhMCP-1. Additionally, in direct ELISA results, this antibody shows no cross-reactivity with other cytokines tested.[1]

Neutralization of Human MCP-3 Bioactivity

The exact concentration of antibody required to neutralize rhMCP-3 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 Dose$_{50}$ (ND$_{50}$)** 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.

As shown in figures 1 and 2 on the next page, the ND$_{50}$ for this lot of anti-human MCP-3 antibody was determined to be approximately 30 - 60 μg/mL in the presence of 0.1 μg/mL of MCP-3, using the monocyte chemotaxis assay. The specific conditions are described in the figure legends.

Additional Applications

For direct ELISAs, the antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect human MCP-3. The detection limit for rhMCP-3 is approximately 1.2 ng/well.

For western blot analysis, the antibody can be used at 1 - 2 μg/mL with the appropriate secondary reagents to detect human MCP-3. The detection limit for rhMCP-3 is approximately 2 ng/lane under non-reducing and reducing conditions. Because this antibody preparation is a total IgG fraction, complete monospecificity cannot be assumed.

Figure 1

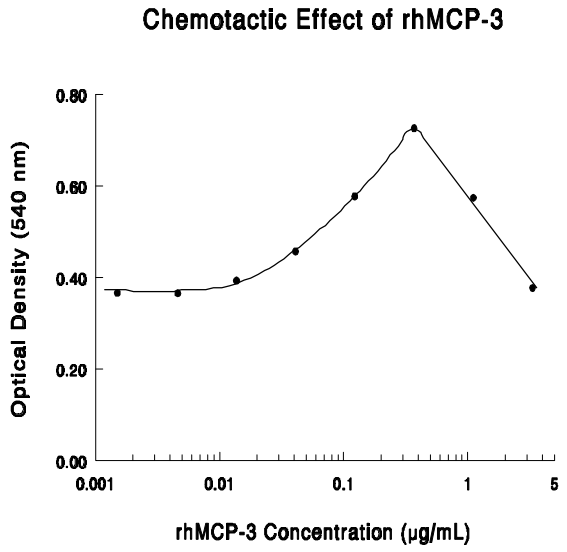

Figure 2

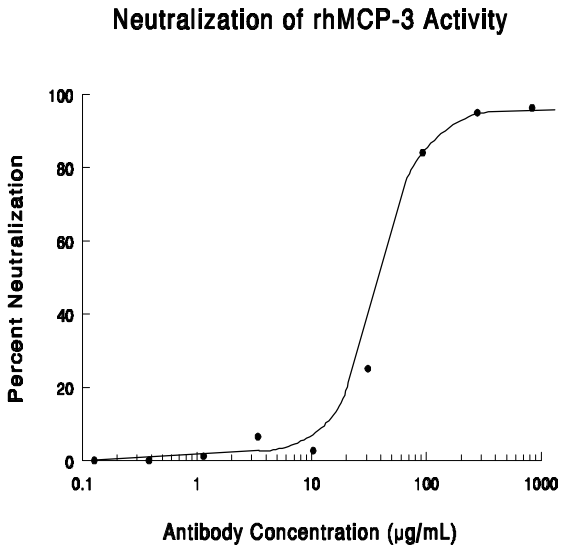

Figure 1

Human MCP-3 chemoattracts human monocytes, exhibiting a bell-shaped dose response curve. The ED_{50} for this effect is typically 0.02 - 0.08 µg/mL.

Figure 2

To measure the ability of the antibody to neutralize the chemoattractant activity of rhMCP-3 on 2 day cultured human monocytes, rhMCP-3 was incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96 well microtiter plate. Following this preincubated period, 35 µL of the cytokine-antibody solution (containing rhMCP-3 at a final concentration of 0.1 µg/mL and antibody at the concentrations indicated) was transferred to the lower compartment of a 96-well chemotaxis chamber (NeuroProbe, Cabin John, MD). The chemotaxis chamber was then assembled using a PVP-free polycarbonate filter (8 micron pore size) and 1 x 10^6 cells/well was added to the top chamber. After incubation for 75 minutes at 37° C in a 5% CO_2 humidified incubator, the chamber was disassembled and the filter was fixed and stained using Leukostat (Fisher Scientific). The optical density of the filter, which is proportional to the number of cells that migrated across the filter, was then read in a microtiter plate reader set at 540 nm. As shown in Figure 2, the ND_{50} for this lot of antibody is approximately 30 - 60 µg/mL.

[1]rhANG, rhAR, rhB7-1, rhB7-2, rmB7-2, rhBTC, rhβ-NGF, rhBDNF, rmC10, rhCD4, rhCD8, rhCD28, rhCNTF, rrCNTF, rhEGF, rhENA-78, rhEpo, rhFGF acidic, rhFGF basic, rhFGF-4, rhFGF-5, rhFGF-6, rhFGF-7, rhFGF-9, rhG-CSF, rmG-CSF, rhGM-CSF, rhGM-CSF Rα, rmGM-CSF, rhGROα, rhGROβ, rhGROγ, rhHB-EGF, rhHRG-α, rhHGF, rhI-309, rhIFN-γ, rhIGF-I, rhIGF-I R, rhIGF-II, rhIL-1α, rhIL-1 RI, rhIL-1 RII, rmIL-1α, rhIL-1β, rmIL-1β, rhIL-1ra, rmIL-1ra, rhIL-2, rhIL-2 sRα, rhIL-2 sRβ, rhIL-2sRγ, rmIL-2, rhIL-3, rhIL-3 sRα, rmIL-3, rhIL-4, rhIL-4 sR, rmIL-4, rhIL-5, rhIL-5 sRα, rhIL-5 sRβ, rmIL-5, rhIL-6, rhIL-6 sR, rmIL-6, rhIL-7, rhIL-7 R, rmIL-7, rhIL-8, rhIL-9, rmIL-9, rhIL-10, rhIL-10 sR, rmIL-10, rhIL-11, rhIL-12, rmIL-12, rhIL-13, rmIL-13, rhIL-15, rhIP-10, rhJAK-1, rmJAK-1, rmJE, rhLIF, rhLIF R, rmLIF, rhM-CSF, rmM-CSF, rhMCP-1 R, rhMidkine, rhMIP-1α, rmMIP-1α, rhMIP-1β, rmMIP-1β, rmMIP-2, rhNT-3, rhNT-4, rhOSM, rhPD-ECGF, hPDGF, pPDGF, rhPDGF-AA, rhPDGF-AB, rhPDGF-BB, rhPDGF Rα, rhPIGF, rhPTN, rhRANTES, rhSCF, rmSCF, rhsgp130, rhSLPI, rhSTAT-1, rmSTAT-4, hTfR, rhTGF-α, rhTGF-β1, rhTGF-β2, rhTGF-β3, raTGF-β5, rhLAP (TGF-β1), rhLatent TGF-β1, rhTGF-β sRII, rhTGF-β RIII, rhTNF-α, rmTNF-α, rhTNF-β, rhsTNF RI, rhsTNF RII, rhTPO, rhVEGF

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