Product Information

Monoclonal Anti-Monocyte Chemotactic Protein-1 Clone 24822

Produced in Mouse, Purified Immunoglobulin

M2420

Product Description

Monoclonal Anti-Human Monocyte Chemotactic Protein-1 (MCP-1/CCL2; IgG1 isotype) is purified from a mouse hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice. Recombinant human MCP-1/CCL2, expressed in *E. coli* was used as immunogen. The antibody is purified by Protein A affinity chromatography.

Monoclonal Anti-Human Monocyte Chemotactic Protein-1 recognizes human MCP-1/CCL2 by immunoblotting and neutralization. The antibody will not neutralize the activity of recombinant human RANTES, recombinant human MIP-1α, or recombinant mouse MIP-1α. When immobilized on a microplate, the antibody will capture both recombinant as well as natural human MCP-1/CCL2.

Monocyte Chemotactic Protein-1 (MCP-1) is also known as Monocyte Chemotactic and Activating Factor (MCAF) and CCL2. MCP-1 is the product of the human JE gene.[1] The MCP-1 gene contains potential binding sites for several transcription factors, including AP-1, AP-2, NF-kB, and NF-IL6.[2] The mature form of MCP-1 has 4 cysteine residues.[3] The first two cysteine residues are in an adjacent position C-C, which characterizes MCP-1 as a member of the chemokine β subfamily. Recombinant human MCP-1/CCL2 is a non-glycosylated protein consisting of 76 amino acids with a molecular mass of 8.7 kDa. MCP-1 mRNA expression can be induced in monocytes/macrophages, B lymphocytes, endothelial cells, and astrocytoma cells by LPS.[2]

IL-1 will induce production of MCP-1 in fibroblasts, keratinocytes, hepatoma cells, and Type II pneumocytes.[2] *In vitro*, MCP-1 will act on monocytes to initiate chemotaxis, induce superoxide anion release, induce the release of lysosomal enzymes, and augment cytostatic activity.[2] *In vivo*, MCP-1 will induce macrophage infiltration.[2]

Reagent

Lyophilized from 0.2 µm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile-filtered phosphate buffered saline to produce a 0.5 mg/mL stock solution of the antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability

Prior to reconstitution, store at −20 °C to −70 °C as supplied. Reconstituted product may be stored at 2-8 °C. For prolonged storage, freeze in working aliquots at −20 °C. Avoid repeated freezing and thawing.

Product Profile

Neutralization

Monoclonal Anti-Monocyte Chemotactic Protein-1 is tested for its ability to neutralize the chemoattractant activity of recombinant human MCP-1/CCL2 for human CCR2A transfected BaF/3 cells.

The exact concentration of antibody required to neutralize recombinant human CCL2/MCP-1 activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

The ND_{50} of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of bioactivity of the cytokine that was present at a concentration just high enough to elicit a maximum response.

1. In the neutralization bioassay, recombinant human CCL2 (MCP-1) was preincubated with various dilutions of the antibody for 30 minutes at room temperature in a 96 well plate.
2. Then, 75 µL of the cytokine-antibody solution (containing recombinant human CCL2 at a final concentration of 0.15 µg/mL) was transferred to the lower compartment of a 96-well chemotaxis chamber.
3. The chemotaxis chamber was then assembled using a PVP-free polycarbonate filter (5-micron pore size) and 0.4 x 10^6 cells/well was added to the top chamber.
4. After incubation for 3 hours at 37 °C in a 5% CO_2 humidified incubator, the chamber was disassembled and the cells that migrated through to the lower chamber were transferred to a spectrofluorometer with excitation wavelength set at 544 nm and emission at 590 nm.

Immunoblotting

A working antibody concentration of 1 µg/mL is recommended. The detection limit for recombinant human MCP-1 is 25 ng/lane under reducing conditions. Chemiluminescent detection will increase sensitivity by 5 to 50-fold.

Note: In order to obtain the best results in various techniques and preparations, it is recommended to determine the optimal working dilutions by titration.

Endotoxin level is <0.1 EU (endotoxin units) per 1 µg antibody as determined by the LAL (Limulus Amebocyte Lysate) method.

References

1. Miller, M. et al., Critical Reviews in Immunology, 12, 17 (1992).
2. Furutani, Y. et al., Biochem. Biophys. Res. Commun., 159, 249 (1989).
3. Mukaida, N. et al., Microbiol. Immunol., 36, 773 (1992).

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