Anti-Macrophage Inflammatory Protein-1β produced in goat, IgG fraction of antiserum

Catalog Number M6792

Product Description
Anti-Macrophage Inflammatory Protein-1β (MIP-1β) is produced in goat using as the immunogen purified, recombinant human macrophage inflammatory protein-1β, expressed in Sf21 insect cells. The antibody is purified by Protein G affinity chromatography.

Anti-Macrophage Inflammatory Protein-1β recognizes recombinant human MIP-1β by immunoblotting, ELISA, and neutralization. The antibody neutralizes the bioactivity of recombinant human MIP-1β. It will not neutralize recombinant mouse MIP-1α, recombinant human MIP-1α, or recombinant mouse MIP-1β.

Macrophage Inflammatory Protein-1β (MIP-1β) belongs to the chemokine β family. In vitro, MIP-1β stimulates H₂O₂ production in human neutrophils.¹

Reagent
Supplied as a lyophilized powder from a 0.2 µm filtered solution of phosphate buffered saline.

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

Preparation Instructions
To one vial of lyophilized powder, add 1 mL of sterile phosphate buffered saline to produce a 1 mg/mL concentration.

Storage/Stability
Prior to reconstitution, store at −20 °C. Reconstituted antibody may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at −20 °C. Avoid repeated freezing and thawing. Do not store in frost-free freezer.

Procedure
To measure the ability of the antibody to neutralize the chemoattractant activity of rhMIP-1β for BaF/3 hCCR5 transfected cells, rhMIP-1β is incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96 well plate. Following this preincubation, 75 µL of the cytokine-antibody mixture (containing rhMIP-1β at a final concentration of 40 ng/mL and antibody at concentrations of 0.1-100 µg/mL) is transferred to the lower compartment of a 96 well chemotaxis chamber. The chamber is then assembled using a PVP-free polycarbonate filter (5 µm pore size) and 0.25 x 10⁶ cells/well is added to the top chamber. After incubation for 3 hours at 37 °C in a 5% CO₂ incubator, the chamber is disassembled. The cells that migrate through to the lower chamber are transferred to a 96 well plate. Chemotaxis is measured by Alamar blue staining of cells that have migrated through the filter.

Product Profile
Anti-Macrophage Inflammatory Protein-1β has the ability to neutralize the biological activity of recombinant human MIP-1β. In a neutralizing bioassay when recombinant human MIP-1β is present at 200 ng/mL, the biological activity can be measured by its ability to inhibit hematopoietic stem cell proliferation in an in vitro colony assay (CFU-A) that detects primitive cells.² Also, a neutralization assay using BaF/3 hCCR5 transfected cells and recombinant human MIP-1β at 0.04 µg/mL maybe used to determine the ND₅₀.

The ND₅₀ is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize recombinant human MIP-1β activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.
Immunoblotting: a working concentration of 1-2 µg/mL is recommended. The detection limit for recombinant human MIP-1β is ~25 ng/lane under non-reducing and reducing conditions. Because this antibody preparation is a total IgG fraction, complete monospecificity cannot be assumed.

ELISA: a working concentration of 0.5-1.0 µg/mL is recommended. The detection limit for recombinant human MIP-1β is ~0.6 ng/well.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining the optimal working dilutions by titration.

Endotoxin level is <10 ng/mg antibody as determined by the LAL (Limulus amebocyte lysate) method.

References