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# **ProductInformation**

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

Catalog Number M6792

## **Product Description**

Anti-Macrophage Inflammatory Protein-1 $\beta$  (MIP-1 $\beta$ ) is produced in goat using as the immunogen purified, recombinant human macrophage inflammatory protein-1 $\beta$ , expressed in *Sf* 21 insect cells. The antibody is purified by Protein G affinity chromatography.

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

Macrophage Inflammatory Protein-1 $\beta$  (MIP-1 $\beta$ ) belongs to the chemokine  $\beta$  family. *In vitro*, MIP-1 $\beta$  stimulates  $H_2O_2$  production in human neutrophils.<sup>1</sup>

#### 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-1ß for BaF/3 hCCR5 transfected cells, rhMIP-1ß 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-1ß at a final concentration of 40 ng/mL and antibody at concentrations of 0.1-100 ug/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 10<sup>6</sup> cells/well is added to the top chamber. After incubation for 3 hours at 37 °C in a 5% CO<sub>2</sub> 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-1 $\beta$  has the ability to neutralize the biological activity of recombinant human MIP-1 $\beta$ . In a neutralizing bioassay when recombinant human MIP-1 $\beta$  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. Also, a neutralization assay using BaF/3 hCCR5 transfected cells and recombinant human MIP-1 $\beta$  at 0.04  $\mu$ g/mL maybe used to determine the ND<sub>50</sub>.

The  $ND_{50}$  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-1 $\beta$  activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working concentration of 1-2  $\mu$ g/mL is recommended. The detection limit for recombinant human MIP-1 $\beta$  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  $\mu$ g/mL is recommended. The detection limit for recombinant human MIP-1 $\beta$  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

- 1. Wolpe, S., et al., *J. Exp. Med.*, **167**, 570 (1988).
- 2. Graham, G., et al., Nature, 344, 442 (1990).

KAA, PHC 05/07-1