

#### **ORDERING INFORMATION**

Catalog Number: MAB1181

Clone: 132016

Lot Number: EPZ02

**Size:** 500 μg

Formulation: 0.2 µm filtered solution of 5%

trehalose in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rhlL-18 Rß

Immunogen: NS0-derived rhIL-18  $R\beta$ 

extracellular domain

Ig class: mouse IgG,

**Applications:** Neutralization of bioactivity

ELISA Western blot

# Monoclonal Anti-human IL-18 Rβ/IL-1 R7 Antibody

## 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, NS0-derived, recombinant human interleukin 18 receptor beta (rhIL-18 R $\beta$ ) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. IL-18 R $\beta$  is alternatively known as IL-1 R7 and Accessory protein-like (AcPL).

#### **Formulation**

Lyophilized from a 0.2  $\mu m$  filtered solution of 5% trehalose in phosphate-buffered saline (PBS) with 5% trehalose.

# 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 500  $\mu$ g/mL.

### **Storage**

Lyophilized samples are stable for greater than six months when held at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for at least 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 at least six months without detectable loss of activity. Avoid repeated freeze-thaw cycles.

## Specificity

This antibody has been selected for its ability to neutralize the biological activity of rhIL-18 R $\beta$ . In direct ELISAs and western blots, this antibody shows less than 1% cross-reactivity with rmIL-18 R $\beta$ , rhIL-18 R, rhIL-1 RI, rhIL-1 RII, rhIL-1 RACP and rhIL-1 Rrp2.

#### Neutralization of Human IL-18 R\$Bioactivity

The exact concentration of antibody required 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**<sub>50</sub> (**ND**<sub>50</sub>) 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 ND $_{50}$  for this lot of anti-human IL-18 R $\beta$  antibody was determined to be approximately 0.3 - 1.0  $\mu$ g/mL in the presence of 40 ng/mL of rhIL-18 and KG-1 cells at 1 x 10 $^6$  cells/mL. The specific conditions are described in the figure legends.

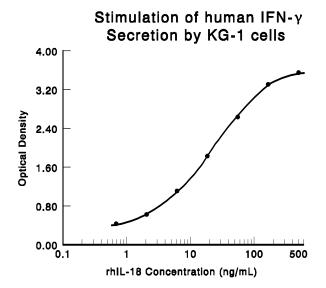
## Additional Applications

Western blot - The antibody can be used at 1 - 2  $\mu$ g/mL with the appropriate secondary reagents to detect human IL-18 R $\beta$ . The detection limit for rhIL-18 R $\beta$  is approximately 5 ng/lane and 50 ng/lane under non-reducing and reducing conditions, respectively.

**Direct ELISA -** This antibody can be used at 0.5 - 1.0  $\mu$ g/mL with the appropriate secondary reagents to detect human IL-18 R $\beta$ . The detection limit for rhIL-18 R $\beta$  is approximately 3 ng/well.

Optimal dilutions should be determined by each laboratory for each application.

R&D Systems, Inc. 1-800-343-7475 Figure 1 Figure 2



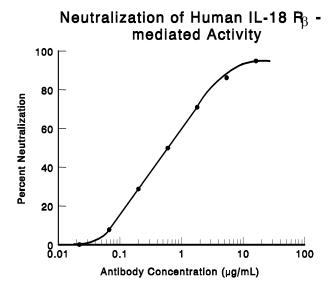


Figure 1 Human IL-18 stimulates IFN- $\gamma$  secretion by KG-1 cells (Novick, D. *et al.*, 1999, Immunity Jan. 10(1):127 - 136). The ED<sub>50</sub> of this effect is typically 10 - 30 ng/mL.

## Figure 2

To measure the ability of the antibody to neutralize human IL-18 R $\beta$ -mediated activity, various concentrations of the antibody were incubated with TNF- $\alpha$  stimulated human KG-1 cells at 2 x 10 $^{\circ}$  cells/well in a 96 well plate for 1 hour at 37 $^{\circ}$  C. Following this preincubation period, rhIL-18 was added. The assay mixture, in a total volume of 200  $\mu$ L, containing antibody at the concentrations indicated, rhIL-18 at 40 ng/mL, TNF- $\alpha$  at 20 ng/mL and cells at 1 x 10 $^{\circ}$  cells/mL, was incubated at 37 $^{\circ}$  C for 1 day in a humidified CO $_{2}$  incubator. After this incubation, 100  $\mu$ L of supernatant was collected from each well, diluted 1:2 with PBS, and tested for human IFN- $\gamma$  levels using a human IFN- $\gamma$  ELISA kit (R&D Systems, Catalog # DIF00). The ND $_{50}$  of the antibody under these conditions is approximately 0.3 - 1  $\mu$ g/mL.