# *Monoclonal Anti-human IL-9 R Antibody*

**ORDERING INFORMATION**

**Catalog Number:** MAB290

**Clone:** 33423

**Lot Number:** RK01

**Size:** 500 μg

**Formulation:** 0.2 μm filtered solution in PBS with 5% trehalose

**Storage:** -20° C

**Reconstitution:** sterile PBS

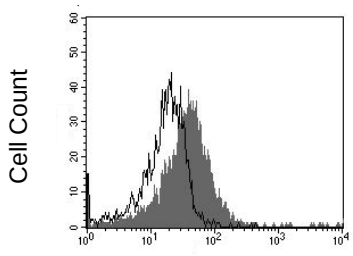
**Specificity:** human IL-9 R

**Immunogen:** *Sf* 21-derived rhIL-9 R extracellular domain

**Ig class:** mouse $IgG_1$

**Recommended Application:**
Neutralization of bioactivity
Flow cytometry

**Other Application:**
Direct ELISA




IL-9 R

Blood-derived monocytes were stained with anti-IL-9 R (R&D Systems, Cat. # MAB290, filled histogram) or isotype control antibody (R&D Systems, Cat. # MAB002, open histogram) followed by PE-conjugated anti-mouse IgG (R&D Systems, Cat. # F0102B).

## *Background*

Interleukin 9 Receptor (IL-9 R) belongs to the hematopoietin receptor superfamily and is the binding subunit of the heterodimeric IL-9 receptor complex. The other subunit is the common γ chain shared with the receptors for IL-2, IL-4, IL-7, and IL-15. IL-9 R is expressed by T cells, neutrophils, mast cells, and macrophages.

## *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, *Sf* 21-derived, recombinant human IL-9 R (rhIL-9 R; aa 40 - 270; Accession # NP_002177) extracellular domain. The IgG fraction of the ascites fluid was purified by Protein G affinity chromatography.

## *Formulation*

Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

## *Endotoxin Level*

< 0.1 EU per 1 μg 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 μg/mL.

## *Storage*

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

## *Specificity*

This antibody detects human IL-9 R. In direct ELISAs, this antibody does not cross-react with rhIL-4 R, rhIL-5 Rα, rhIL-5 Rβ, rhIL-13 Rα1, rhIL-13 Rα2, or rmIL-9 R.

## *Applications*

**Neutralization of Human IL-9 Receptor-mediated bioactivity -** The exact concentration of antibody required to neutralize the human cell surface IL-9 R mediated bioactivity is dependent on the IL-2 concentration as well as on the number and types of IL-2 receptors present on the cell surface (a function of cell type and culture conditions). 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 cell surface IL-9 R mediated IL-9 response on a responsive cell line, at a specific IL-9 concentration.

As shown in figures 1 and 2 on the next page, the $ND_{50}$ for this lot of anti-human IL-9 R antibody was determined to be approximately 2 - 4 μg/mL for inhibiting the IL-9-dependent proliferation of MO7e cells (1 x $10^5$ cells/mL) in the presence of 2 ng/mL of rhIL-9. The specific conditions are described in the figure legends.

**Flow cytometry -** This antibody was validated for flow cytometry using blood-derived monocytes. Dilute this antibody to 50 μg/mL and add 10 μL of the diluted solution to 1-2.5 x $10^5$ cells in a total reaction volume not exceeding 200 μL. The binding of unlabeled antibodies may be visualized by adding a secondary developing reagent such as anti-mouse IgG conjugated to a fluorochrome.

**Direct ELISA -** This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect human IL-9 R. The detection limit for rhIL-9 R is approximately 6 ng/well.

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



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

**Figure 1**

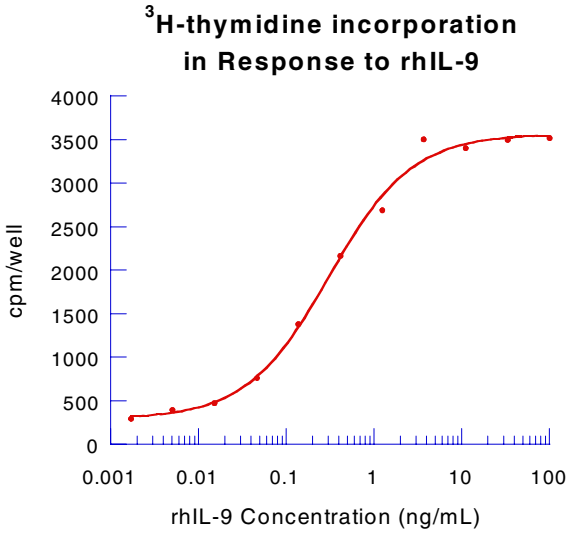



**Figure 2**

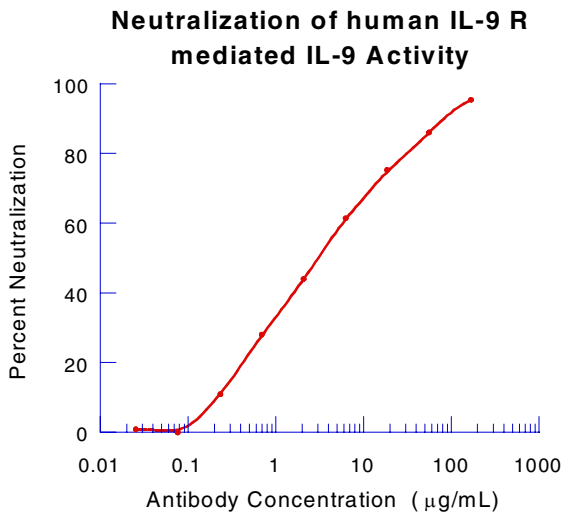



**Figure 1**
Human IL-9 stimulates the 3H-thymidine incorporation by MO7e cells in a dose-dependent manner (Avanzi, G. *et al*., 1988, British Journal of Haematology **69**:359 - 366). The $ED_{50}$ for this effect is typically 0.5 - 1.0 ng/mL.

**Figure 2**
To measure the ability of the antibody to block the IL-9 R mediated IL-9 response on human MO7e cells, various concentrations of IL-9 R antibody were incubated with MO7e cells for 1 hour at 37° C in a 96 well microtiter plate. Following this incubation, rhIL-9 was added to the plate. The assay mixture, in a total volume of 100 μL, containing MO7e cells at 1 x $10^5$ cells/mL, rhIL-9 at 2 ng/mL, and hIL-9 R antibody at the concentrations indicated, was incubated for 72 hours at 37° C in a 5% $CO_2$ humidified incubator. Cells were pulsed with $^3$H-thymidine for the final 4 hours and then harvested onto glass fiber filters, and the $^3$H-thymidine incorporated into DNA was determined. The $ND_{50}$ of the antibody is approximately 2 - 4 μg/mL.