

ORDERING INFORMATION

Catalog Number: MAB272

Clone: 35305

Lot Number: AYW03

Size: 500 µg

Formulation: 0.2 µm filtered solution of 5% trehalose in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human I-309

Immunogen: *E. coli*-derived rhl-309

Ig class: mouse IgG₁

Recommended Applications:
Neutralization of bioactivity
Western blot
ELISA capture

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, *E. coli*-derived, recombinant human I-309 (rhl-309). The IgG fraction of ascites fluid was purified by Protein A affinity chromatography. I-309 is a member of the CC chemokine family and has been designated CCL1. It signals through the 7-TM receptor, CCR8.

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 was selected for its ability to neutralize the biological activity of rhl-309 and for use as a capture antibody in sandwich ELISAs. This antibody does not neutralize the biological activity of rmTCA-3.

Applications

Neutralization of Human I-309 bioactivity - The exact concentration of antibody required to neutralize rhl-309 activity 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 Dose₅₀ (ND₅₀)** 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 ND₅₀ for anti-human I-309 antibody was determined to be approximately 0.1 - 0.5 µg/mL in the presence of 0.02 µg/mL of rhl-309 using hCCR8 transfected BaF/3 cells in a chemotaxis assay.

Figure 1: Human I-309 chemoattracts hCCR8 transfected BaF/3 cells. The ED₅₀ for this effect is typically 1.5 - 7.5 ng/mL.

Figure 2: Typical data for the anti-human I-309 antibody is shown in Figure 2. Approximately 0.1 - 0.5 µg/mL of the antibody will neutralize 50% of the bioactivity due to 0.02 µg/mL of rhl-309.

Western blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect human I-309. The detection limit for rhl-309 is approximately 1 ng/lane and 50 ng/lane under non-reducing and reducing conditions, respectively. In Western blots, this antibody shows no cross-reactivity with other chemokines tested.¹

ELISA capture - This antibody can be used as a capture antibody in a human I-309 ELISA in combination with biotinylated, human I-309 affinity purified polyclonal detection antibody (Catalog # BAF272). A general protocol is provided on the next page. Using plates coated with 100 µL/well of the capture antibody at 2 µg/mL, in combination with 100 µL/well of the detection antibody, an ELISA for sample volumes of 100 µL can be obtained. To arrive at the optimal dose range for this ELISA, set up a two-fold dilution series of the protein standard starting with 2 ng/mL.

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

¹rh6Ckine, rm6Ckine, rmC10, rhCCL28, rrCINC-1, rhCXC-X, rhENA-78, rhEotaxin, rmEotaxin, rhFractalkine, rhGCP-2, rmGCP-2, rhGROα, rhGROβ, rhGROγ, rhHCC-1, rhIL-8, rhIP-10, rmJE, rmKC, rmMARC, rhMCP-1, rhMCP-2, rhMCP-3, rhMIG, rmMIG, rhMIP-1α, rmMIP-1α, rhMIP-1β, rhMIP-1γ, rmMIP-1γ, rhMIP-3α, rhMIP-3β, rhNAP-2, rhRANTES, rmRANTES, rhSDF-1α, rhSDF-1β, rhTarc, rhTeck, rmTeck

Figure 1

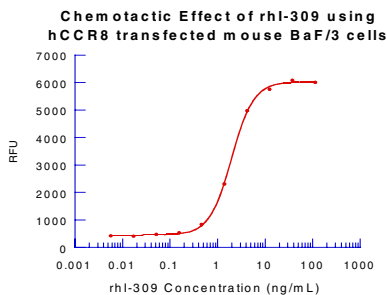


Figure 2

