Anti-human CCL16/HCC-4 Antibody

ORDERING INFORMATION

Catalog Number: AF802

Lot Number: ATR015041

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human HCC-4

Immunogen: *E. coli*-derived rhHCC-4

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA
Immunohistochemistry

Preparation
Produced in goats immunized with purified, *E. coli*-derived, recombinant human HCC-4 (rhHCC-4). HCC-4 specific IgG was purified by human HCC-4 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 0.5 mL of PBS is used, the antibody concentration will be 0.2 mg/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 has been selected for its ability to neutralize rhHCC-4 bioactivity. Based on direct ELISA and western blot results, this antibody shows less than 5% cross-reactivity with rmCRG-2. Additionally, in direct ELISA, this antibody shows no cross-reactivity with other chemokines tested.[1]

Neutralization of Human HCC-4 Bioactivity
The exact concentration of antibody required to neutralize rhHCC-4 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 Dose$_{50}$ (ND$_{50}$)** 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 HCC-4 antibody was determined to be approximately 4.0 - 16.0 μg/mL in the presence of 0.75 μg/mL of rhHCC-4, using BaF/3 cells transfected with the hCCR1 receptor. The specific conditions are described in the figure legends.

Additional Applications
Direct ELISAs - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect human HCC-4. The detection limit for rhHCC-4 is approximately 0.16 ng/well.

Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect human HCC-4. The detection limit for rhHCC-4 is approximately 2 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry - This antibody will detect HCC-4 in paraffin-embedded human tissue sections. The working dilution for 5 - 15 μm thick sections is 15 μg/mL. For chromogenic detection of labeling, it is recommended to use R&D Systems' Cell and Tissue Staining kits (CTS Series).

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

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

Figure 1

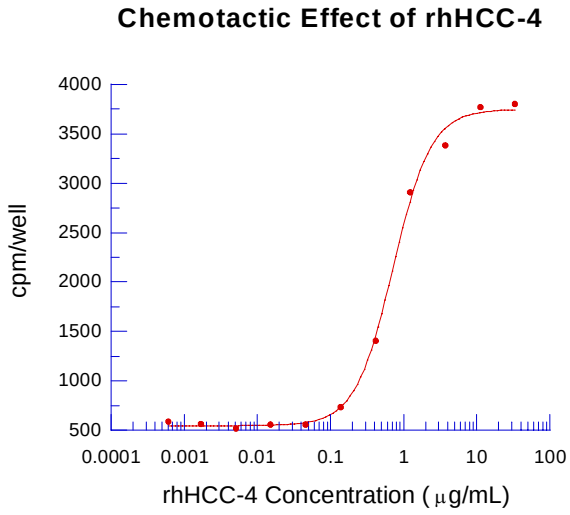

Figure 2

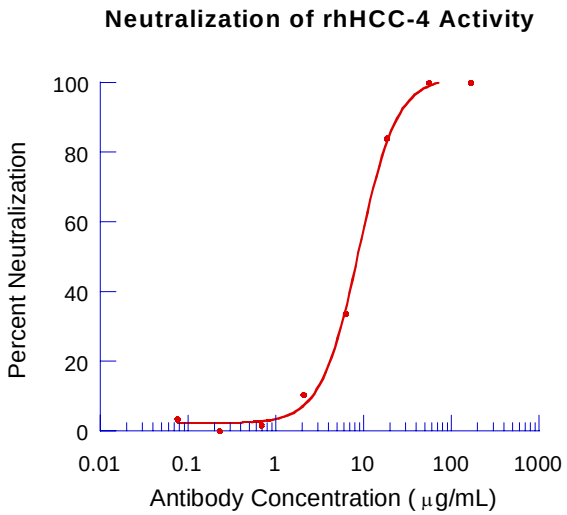

Figure 1
Human HCC-4 chemoattracts mouse BaF/3 cells transfected with hCCR1. The ED_{50} for this effect is typically 0.15 - 0.75 μg/mL.

Figure 2
To measure the ability of the antibody to neutralize the chemoattracant activity of rhHCC-4 for hCCR1 transfected cells, rhHCC-4 was incubated with various concentrations of the antibody for 30 minutes at room temperature in a 96 well microplate. Following this preincubation period, 75 μL of the cytokine-antibody solution (containing rhHCC-4 at a final concentration of 750 ng/mL and antibody at the concentrations indicated) was transferred to the lower compartment of a 96 well chemotaxis chamber (NeuroProbe, Cabin John, MD). The chemotaxis chamber was then assembled using a PVP-free polycarbonate filter (5 micron pore size) and 0.25 x 10^6 cells/well was added to the top chamber. After incubation for 3 hours at 37° C in a 5% CO_2 humidified incubator, the chamber was carefully disassembled. The cells that migrate through to the lower chamber were transferred to a 96 well plate. Chemotaxis was measured by Resazurin (R&D Systems, Catalog # AR002) staining of cells that have migrated through the filter. The ND_{50} for this lot of antibody is approximately 4.0 - 16.0 μg/mL.