Anti-human HCC-1/CCL14a Antibody

ORDERING INFORMATION

Catalog Number: AF-324-PB

Lot Number: ADV0108091

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human HCC-1

Immunogen: *E. coli*-derived rhHCC-1

Ig Type: hHCC-1 specific goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation
Produced in goats immunized with purified, *E. coli*-derived, recombinant human hemofiltrate CC chemokine 1 (rhHCC-1). HCC-1 specific IgG was purified by human HCC-1 afffinity 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 0.1 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-1 bioactivity. Based on direct ELISA and Western blot results (non-reducing conditions), this antibody shows less than 5% cross-reactivity with rhMCP-2, rhMCP-3, rmC10 and rmMIP-1β.

Neutralization of Human HCC-1 bioactivity
The exact concentration of antibody required to neutralize human HCC-1 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-1 antibody was determined to be approximately 0.15 - 0.75 μg/mL in the presence of 10 ng/mL of rhHCC-1 (R&D Systems, Catalog # 1578-HC), using the hCCR1 transfected BaF/3 cell line. The specific conditions are described in the figure legends.

Additional Applications
Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect human HCC-1. The detection limit for rhHCC-1 is approximately 1 ng/lane under non-reducing and reducing conditions.

Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect human HCC-1. The detection limit for rhHCC-1 is approximately 0.16 ng/well.

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

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1-800-343-7475

Figure 1

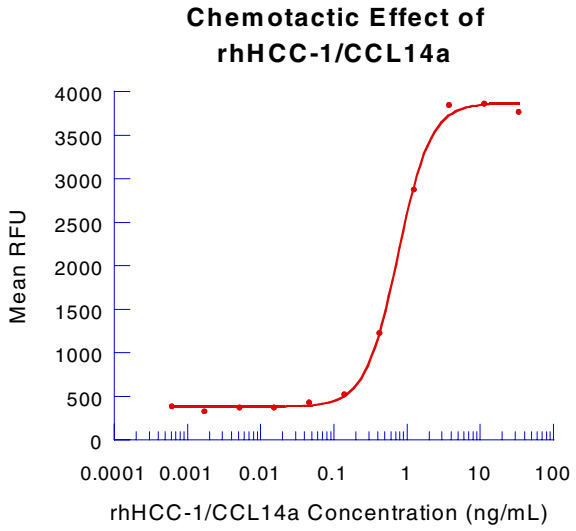

Figure 2

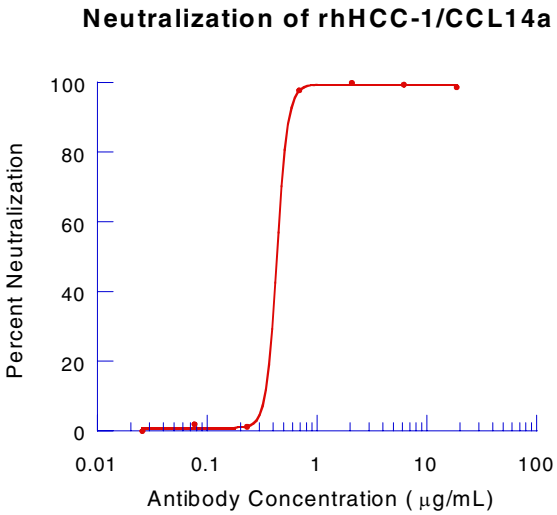

Figure 1
rhHCC-1/CCL14a (R&D Systems, Catalog # 1578-HC) chemoattracts hCCR1 transfected BaF/3 cells. The number of cells that have migrated through to the lower chamber are quantitated using Resazurin (R&D Systems, Catalog # AR002) staining. The ED_{50} for this effect is typically 0.15 - 0.75 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the chemoattractant activity of rhHCC-1 for BaF/3 hCCR1 cells, rhHCC-1/CCL14a (R&D Systems, Catalog # 1578-HC) was incubated with various concentrations of 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-1/CCL14a (R&D Systems, Catalog # 1578-HC) at a final concentration of 10 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 2.5 x 10^5 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 disassembled and the cells that migrated through to the lower chamber were transferred to a working plate and stained using Resazurin (R&D Systems, Catalog # AR002). The relative fluorescence was then read in a 96-well spectrofluorimeter with excitation wavelength set at 544 nm and emission at 590 nm. As shown in Figure 2, the ND_{50} for this lot of antibody is approximately 0.15 - 0.75 µg/mL.