



## ***Anti-cotton rat RANTES/CCL5 Antibody***

### **ORDERING INFORMATION**

**Catalog Number:** AF1010

**Lot Number:** HCD02

**Size:** 100 µg

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

**Storage:** -20° C

**Reconstitution:** sterile PBS

**Specificity:** cotton rat RANTES

**Immunogen:** *E. coli*-derived rcrRANTES

**Ig Type:** cotton rat RANTES specific goat IgG

**Applications:** Neutralization of bioactivity  
Western blot  
ELISA

### ***Preparation***

Produced in goats immunized with purified, *E. coli*-derived, recombinant cotton rat RANTES (rcrRANTES). Cotton rat RANTES specific IgG was purified by cotton rat RANTES 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 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 cotton rat RANTES bioactivity.

### ***Neutralization of Cotton Rat RANTES Bioactivity***

The exact concentration of antibody required to neutralize rcrRANTES 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<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<sub>50</sub> for this lot of anti-cotton rat RANTES antibody was determined to be approximately 1 - 4 µg/mL in the presence of 0.2 µg/mL of rcrRANTES, using the mCCR5 transfected BaF/3 cell chemotaxis assay. The specific conditions are described in the figure legends.

### ***Additional Applications***

**Direct ELISA** - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect cotton rat RANTES. The detection limit for rcrRANTES is approximately 1 ng/well. In this format, this antibody shows less than 20% cross-reactivity with rhRANTES and rmRANTES and less than 1% cross-reactivity with other chemokines tested.<sup>1</sup>

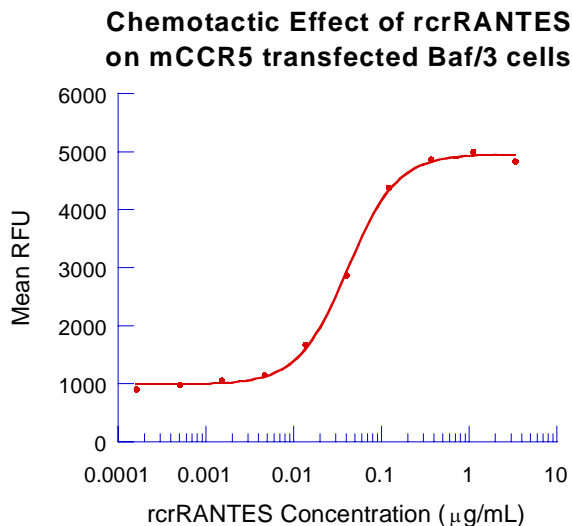
**Western blot** - This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect cotton rat RANTES. The detection limit for rcrRANTES is approximately 10 ng/lane under non-reducing and reducing conditions.

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

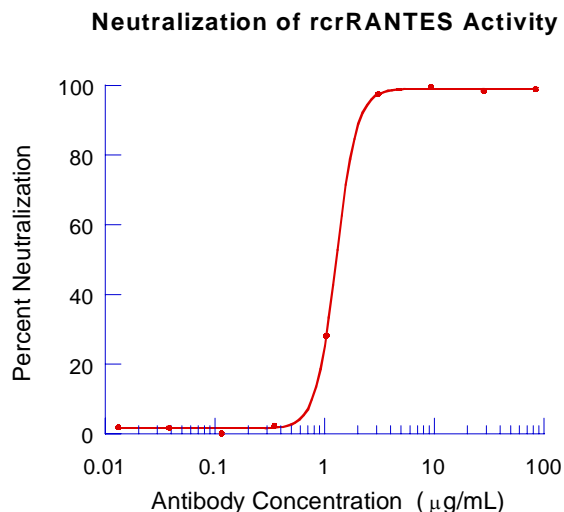
FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

**Figure 1**



**Figure 2**



**Figure 1**

Cotton rat RANTES chemoattracts mCCR-5 transfected BaF/3 cells. The ED<sub>50</sub> for this effect is typically 0.02 - 0.1 µg/mL.

**Figure 2**

To measure the ability of the antibody to neutralize the chemoattractant activity of rcrRANTES for BaF/3 mCCR-5 cells, rcrRANTES 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 rcrRANTES at a final concentration of 0.2 µg/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<sup>6</sup> cells/well was added to the top chamber. After incubation for 3 hours at 37° C in a 5% CO<sub>2</sub> 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 read with excitation wavelength set at 544 nm and emission at 590 nm. As shown in Figure 2, the ND<sub>50</sub> for this lot of antibody is approximately 1 - 4 µg/mL.

<sup>1</sup>rh6Ckine, rm6Ckine, rhBLC/BCA-1, rmBLC, rhBRAK, rmBRAK, rmC10, rhCCL28, rmCCL28, rrCINC-1, rrCINC-2 $\alpha$ , rrCINC-2 $\beta$ , rrCINC-3, rhCK $\beta$ 8-1, rvCMV UL146, rmCRG-2, rmCTACK, rhCXCL16, rmCXCL16, rhENA-78, rhEotaxin, rmEotaxin, rhEotaxin-2, rmEotaxin-2, rhEotaxin-3, rhFractalkine, rmFractalkine, rrFractalkine, rhGCP-2, rmGCP-2, rhGRO $\alpha$ , rhGRO $\beta$ , rhGRO $\gamma$ , rhHCC-1, rhHCC-4, rhl-309, rhlL-8, rplL-8, rcrIP-10, rhIP-10, rhl-TAC, rml-TAC, rmJE, rmKC, rhLeukotactin-1, rrLIX, rmLungkine, rhLymphotactin, rmLymphotactin, rmMARC, rhMCP-1, rhMCP-2, rmMCP-2, rhMCP-3, rhMCP-4, rmMCP-5, rvMCV type 2, rhMDC, rmMDC, rhMIG, rmMIG, rcrMIP-1 $\alpha$ , rhMIP-1 $\alpha$ , rmMIP-1 $\alpha$ , rcrMIP-1 $\beta$ , rhMIP-1 $\beta$ , rmMIP-1 $\beta$ , rhMIP-1 $\delta$ , rmMIP-1 $\gamma$ , rmMIP-2, rhMIP-3 $\alpha$ , rmMIP-3 $\alpha$ , rrMIP-3 $\alpha$ , rhMIP-3 $\beta$ , rmMIP-3 $\beta$ , rvMIP-I, rvMIP-II, rvMIP-III, rhMPIF-1, rhNAP-2, rhPARC, rhPF4, rmPF4, rhSDF-1 $\alpha$ , rmSDF-1 $\alpha$ , rhSDF-1 $\beta$ , rhTARC, rmTARC, rmTCA-3, rhTeck, rmTeck