



# Monoclonal Anti-cotton rat IP-10/CXCL10 Antibody

## ORDERING INFORMATION

**Catalog Number:** MAB1117

**Clone:** 163426

**Lot Number:** HPQ01

**Size:** 500 µg

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

**Storage:** -20° C

**Reconstitution:** sterile PBS

**Specificity:** cotton rat IP-10

**Immunogen:** *E. coli*-derived rcrIP-10

**Ig class:** mouse IgG<sub>1</sub>

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

## 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 cotton rat IP-10 (rcrIP-10). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. IP-10 is a member of the CXC chemokine superfamily and has been designated CXCL10.

## 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 bioactivity of rcrIP-10. In direct ELISAs, this antibody showed no cross-reactivity with other tested chemokines.<sup>1</sup>

## Neutralization of Cotton Rat IP-10 Bioactivity

The exact concentration of antibody required to neutralize rcrIP-10 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 IP-10 antibody was determined to be approximately 20 - 40 µg/mL in the presence of 10 µg/mL of rcrIP-10, using chemoattraction of the mCXCR-3 transfected BaF/3 cell line as an 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 IP-10. The detection limit for rcrIP-10 is approximately 20 ng/well.

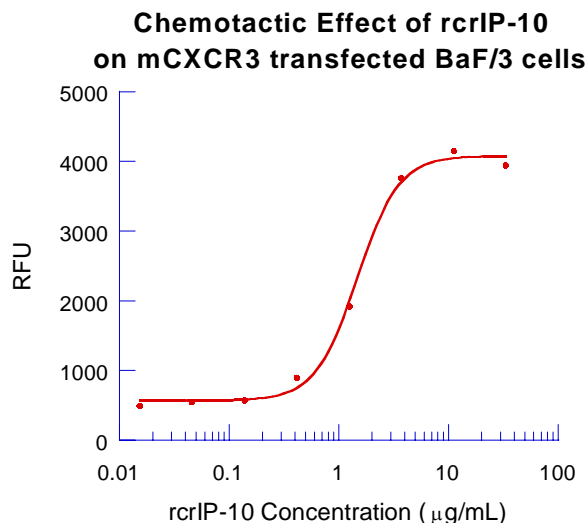
**Western Blot** - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect cotton rat IP-10. The detection limit for rcrIP-10 is approximately 25 ng/lane under non-reducing conditions.

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

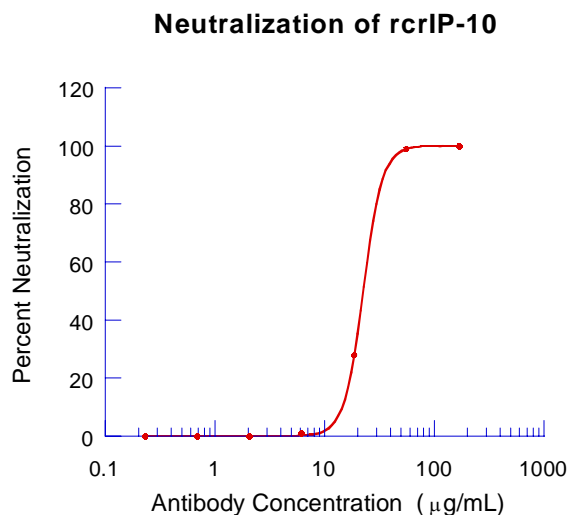
FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

**Figure 1**



**Figure 2**



**Figure 1**

Cotton rat IP-10 chemoattracts BaF/3 cells that have been transfected with mCXCR3. The number of cells that have migrated through to the lower chamber are quantified using Resazurin (R&D Systems Catalog # AR002) staining. The ED<sub>50</sub> for this effect is typically 2 - 10 µg/mL.

**Figure 2**

To measure the ability of the antibody to neutralize the chemoattractant activity of rcrIP-10 for mCXCR3 transfected BaF/3 cells, rcrIP-10 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 rcrIP-10 at a final concentration of 10 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 2.5 x 10<sup>5</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 fluorescence was then read in a microplate reader set at 544/590 nm. As shown in Figure 2, the ND<sub>50</sub> for this lot of antibody is approximately 20 - 40 µg/mL.

<sup>1</sup>rhBLC/BCA-1, rmBLC/BCA-1, rhCCL28, rmCCL28, rh6Ckine, rm6Ckine, rmC10, rrCINC-1, rrCINC-2 $\alpha$ , rrCINC-2 $\beta$ , rrCINC-3, rhCK $\beta$ 8-1, rvCMV UL146, rvCMV UL147, rmCRG-2, rhCTACK, rmCTACK, rhCXCL-16, rmCXCL-16, rhCXC-X3, rmCXC-X3, 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, rhI-309, rhIL-8, rplL-8, rhIP-10, rmIP-10, rhI-TAC, rmI-TAC, rmJE, rmKC, rhLeukotactin-1, rrLIX, rmLungkine, rhLymphotactin, rmLymphotactin, rmMarc, rhMCP-1, rhMCP-2, rmMCP-2, rhMCP-3, rhMCP-4, rhMCP-5, rmMCP-5, rmMCV type 2, rhMDC, rmMDC, rhMIG, rmMIG, rcrMIP-1 $\alpha$ , rhMIP-1 $\alpha$ , rmMIP-1 $\alpha$ , rcrMIP-1 $\beta$ , rmMIP-1 $\beta$ , rhMIP-1 $\beta$  (ACT II), rhMIP-1 $\delta$ , rmMIP-1 $\gamma$ , rmMIP-2, rvMIP-I, rvMIP-II, rhMIP-3 $\alpha$ , rhMIP-3 $\beta$ , rmMIP-3 $\alpha$ , rmMIP-3 $\beta$ , rrMIP-3 $\alpha$ , rvMIP-III, rhPF4, rmPF4, rhMPIF-I, rhNAP-2, rhPARC, rcrRANTES, rhRANTES, rmRANTES, rhSDF-1 $\alpha$ , rhSDF-1 $\beta$ , rmSDF-1 $\alpha$ , rhTarc, rmTarc, rmTCA-3, rhTeck, rmTeck, rmThymusCK1