

## ORDERING INFORMATION

**Catalog Number:** AB-257-NA

**Lot Number:** EM03

**Size:** 1 mg

**Formulation:** 0.2 µm filtered solution in PBS

**Storage:** -20° C

**Reconstitution:** sterile PBS

**Specificity:** human CNTF

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

**Ig Type:** goat IgG

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

## **Preparation**

Produced in goats immunized with purified, *E. coli*-derived, recombinant human ciliary neurotrophic factor (rhCNTF). Total IgG was purified by Protein G affinity chromatography.

## **Formulation**

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS).

## **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 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 the biological activity of rhCNTF.

## **Neutralization of CNTF Bioactivity**

The exact concentration of antibody required to neutralize rhCNTF 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.

As shown in figures 1 and 2 on the next page, the ND<sub>50</sub> for this lot of anti-human CNTF antibody was determined to be approximately 15 - 30 µg/mL in the presence of 10 ng/mL of rhCNTF, in the neuron survival assay using embryonic chick dorsal root ganglia neurons. The specific conditions are described in the figure legends. Using TF-1 cells, the ND<sub>50</sub> of this lot of anti-human CNTF antibody was determined to be approximately 150 - 250 µg/mL in the presence of 400 ng/mL of rhCNTF.

## **Additional Applications**

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

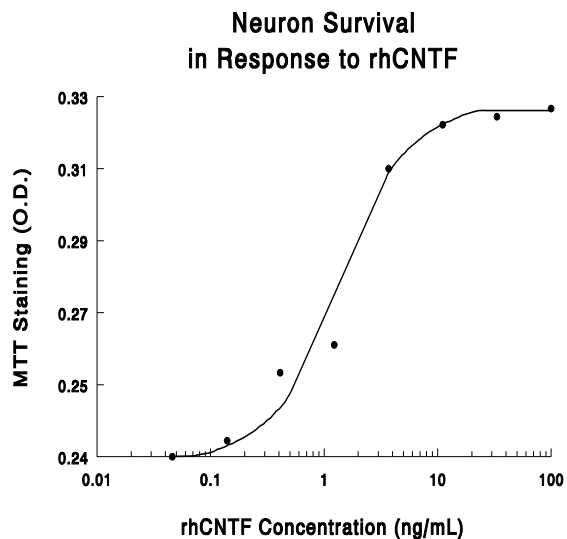
**Western blot** - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect rhCNTF. The detection limit for rhCNTF is approximately 2 ng/lane and 5 ng/lane under non-reducing and reducing conditions, respectively. Because this antibody preparation is a total IgG fraction, complete monospecificity cannot be assumed.

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

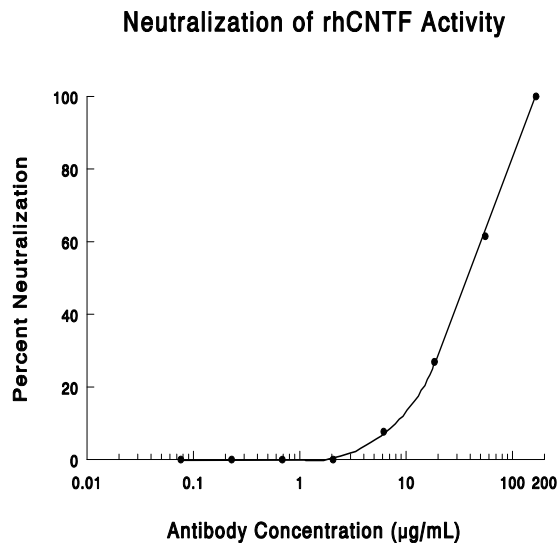
FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

**Figure 1**



**Figure 2**



**Figure 1**

Human CNTF supports the DRG neuron survival in a dose-dependent manner (Davies, A.M., 1989, "Neurotrophic Factor Bioassay Using Dissociated Neurons" in *Nerve Growth Factors*, R.A. Rush Ed., John Wiley and Sons, Ltd. pp. 95-109). The ED<sub>50</sub> for this effect is typically 1 - 3 ng/mL.

**Figure 2**

To measure the neutralizing activity of this antibody, various concentrations of rhCNTF antibody were incubated with rhCNTF for 1 hour at 22° C in a poly-DL-ornithine and laminine precoated 96 well microtiter plate. Following this incubation period, DRG neurons from day 10 chick embryos were added to the plate. The assay mixture, in a total volume of 100 µL, containing rhCNTF at 10 ng/mL, neurons at  $1 \times 10^5$  cells/mL and hCNTF antibody at the concentrations indicated, was incubated at 37° C for 72 hours in a 5% CO<sub>2</sub> humidified incubator. Neuron survival was measured by MTT staining. The ND<sub>50</sub> of the antibody is approximately 15 - 30 µg/mL.