# Anti-human CNTF Ra Neutralizing Antibody

**ORDERING INFORMATION**

**Catalog Number:** AF-303-NA

**Lot Number:** AAG01

**Size:** 100 µg

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

**Storage:** -20° C

**Reconstitution:** sterile PBS

**Specificity:** rhCNTF Rα

**Immunogen:** *Sf* 21 cell-derived rhCNTF sRα

**Ig class:** hCNTF Rα extracellular domain-specific goat IgG

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

## Preparation

Produced in goats immunized with purified, *Sf* 21-derived, recombinant human ciliary neurotrophic factor soluble receptor alpha (rhCNTF sRα). CNTF Rα specific IgG was purified by human CNTF Rα affinity chromatography.

## Formulation

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

## Endotoxin Level

< 10 ng per 1 mg 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 100 µg/mL.

## Storage

Lyophilized samples are stable for greater than 6 months at -20° C to -70° C. Reconstituted antibody is stable for at least 1 month at 2° - 4° C or 3 months at -20° C to -70° C under sterile conditions. **Avoid repeated freeze-thaw cycles.**

## Specificity

This antibody has been selected for its ability to block recombinant human CNTF sRα mediated bioactivities. Based on western blot results, this antibody shows greater than 50% cross-reactivity with rrCNTF sRα and less than 5% cross-reactivity with rhIL-2 sRγ and rhIL-6 sR. Additionally, in direct ELISA, this antibody does not cross-react with other cytokines tested.[1]

## Neutralization of Human CNTF Ra Bioactivity

The exact concentration of antibody required to neutralize rhCNTF sRα 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 CNTF Rα antibody was determined to be approximately 10 - 20 µg/mL in the presence of 1 µg/mL of rhCNTF sRα and 20 ng/mL of rhCNTF, using the TF1 cell line. The specific conditions are described in the figure legends.

## Additional Applications

For direct ELISAs, the antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human CNTF sRα. The detection limit for rhCNTF sRα is approximately 0.06 ng/well.

For western blot analysis, the antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect human CNTF sRα. The detection limit for rhCNTF sRα is approximately 5 ng/lane under non-reducing and reducing conditions.



**R&D Systems Inc.**

**1-800-343-7475**

**Figure 1**

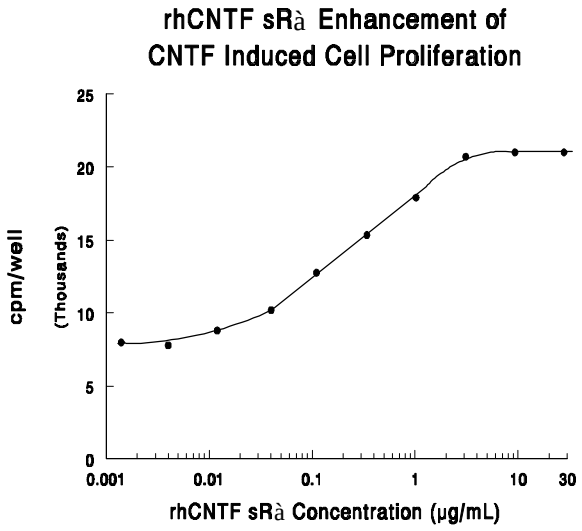


**Figure 2**

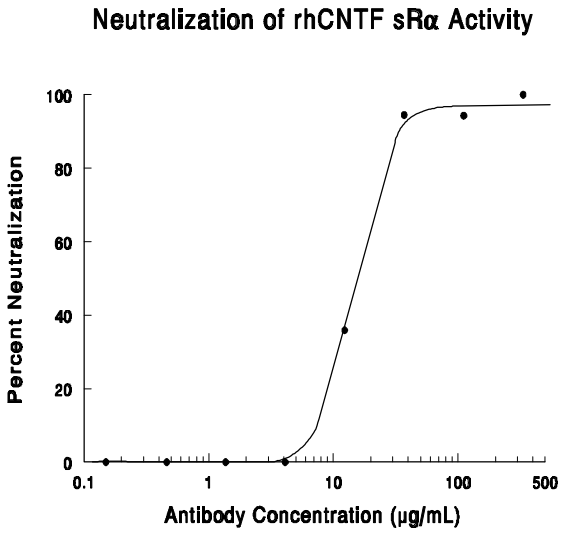


**Figure 1**
rhCNTF sRα enhances CNTF-dependent proliferation of human TF-1 cells in a dose-dependent manner. (Kitamura, T. *et al*., 1989, J. Cell Physiol. **140**:323-334). The $ED_{50}$ for this effect is typically 0.2 - 0.4 µg/mL in the presence of 20 ng/mL of rhCNTF.

**Figure 2**
Typical data for anti-hCNTF Rα is shown in Figure 2. To measure the ability of the antibody to block rhCNTF sRα response on TF-1 cells, rhCNTF sRα was incubated with serial dilutions of the antibody for 1 hour at 37° C in a 96 well microplate. Following this preincubation, rhCNTF and TF-1 cells are added. The assay mixture in a total volume of 100 µL, containing antibody at the concentrations indicated, rhCNTF sRα at 1 µg/mL, rhCNTF at 20 ng/mL and cells at 1 X $10^5$ cells/mL, was incubated at 37° C for 48-72 hours in a humidified $CO_2$ incubator. $^3$H-thymidine was added during the final 4 hours of incubation. The cells were subsequently harvested onto glass fiber filters and the $^3$H-thymidine incorporated into DNA was determined. The $ND_{50}$ of the antibody is approximately 10 - 20 µg/mL.

[1]rhANG, rhAnnexin V, rhAR, rhB7-1, rhB7-2, rmB7-2, rhBTC, rhβ-NGF, rhBDNF, rmC10, rhCD4, rhCD8, rhCD28, rhCNTF, rrCNTF, rhEGF, rhENA-78, rhEotaxin, rmEotaxin, rhEPO, rhEPO R, rhFGF acidic, rhFGF basic, rhFGF-4, rhFGF-5, rhFGF-6, rhFGF-7, rhFGF-9, rhFlk2/Flt3, rhG-CSF, rhG-CSF Rα, rmG-CSF, rhGDNF, rrGDNF, rhGM-CSF, rhGM-CSF Rα, rmGM-CSF, rhGROα, rhGROβ, rhGROγ, rhHB-EGF, rhHRG-α, rhHGF, rhI-309, rhIFN-γ, rmIFN-γ, rhIGF-I, rhIGF-I R, rhIGF-II, rhIL-1α, rhIL-1 RI, rhIL-1 RII, rmIL-1α, rhIL-1β, rmIL-1β, rrIL-1β, rhIL-1ra, rmIL-1 ra, rhIL-2, rhIL-2 sRα, rhIL-2 sRβ, rmIL-2, rrIL-2, rhIL-3, rhIL-3 sRα, rmIL-3, rhIL-4, rhIL-4 sR, rmIL-4, rrIL-4, rhIL-5, rhIL-5 sRα, rmIL-5, rhIL-6, rmIL-6, rhIL-7, rhIL-7 R, rmIL-7, rhIL-8, rhIL-9, rhIL-9 sR, rmIL-9, rhIL-10, rhIL-10 sR, rmIL-10, rmIL-10 sR, rhIL-11, rmIL-11, rhIL-12, rmIL-12, rhIL-13, rmIL-13, rhIL-15, rhIL-16, rhIL-17, rhIP-10, rmJAK-1, rmJAK-2, rmJE, rmKC, rhLIF, rhLIF R, rmLIF, rmLymphotactin, rhM-CSF, rmM-CSF, rhMCP-1, rhMCP-1 R, rhMCP-2, rhMCP-3, rhMidkine, rhMIF, rmMIG, rhMIP-1α, rmMIP-1α, rhMIP-1β, rmMIP-1β, rmMIP-2, rhNT-3, rhNT-4, rmOB, rhOSM, rmOSM, rhPD-ECGF, hPDGF, pPDGF, rhPDGF-AA, rhPDGF-AB, rhPDGF-BB, rhPDGF Rα, rhPlGF, rhPTN, rhRANTES, rmRANTES, rhSCF, rmSCF, rhsgp130, rhSLPI, rhSTAT-1, rmSTAT-4, hTfR, rhTGF-α, rhTGF-β1, rhTGF-β2, rhTGF-β3, raTGF-β5, rhLAP (TGF-β1), rhLatent TGF-β1, rhTGF-β sRII, rhTGF-β sRIII, rhTNF-α, rmTNF-α, rrTNF-α, rhTNF-β, rhsTNF RI, rhsTNF RII, rhTPO, rmTPO, rhTyk-2, rhVEGF, rhVEGF/PlGF, rmVEGF

8/26/97