Anti-human NT-4 Antibody

ORDERING INFORMATION

Catalog Number: AF-268-NA

Lot Number: VK02

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human NT-4

Immunogen: *Sf* 21-derived rhNT-4

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Immunohistochemistry
Direct ELISA

Preparation
Produced in goats immunized with purified, insect cell line *Sf* 21-derived, recombinant human neurotrophin 4 (rhNT-4). NT-4 specific IgG was purified by NT-4 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. Avoid repeated freeze-thaw cycles.**

Specificity
This antibody has been selected for its ability to neutralize the biological activity of human NT-4. In direct ELISAs and western blots, this antibody shows less than 1% cross-reactivity with rhNT-3 and rhBDNF.

Neutralization of Human NT-4 Bioactivity
The exact concentration of antibody required to neutralize rhNT-4 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.

As shown in figures 1 and 2 on the next page, the ND$_{50}$ for this lot of anti-human NT-4 antibody was determined to be approximately 1 - 3 µg/mL in the presence of 40 ng/mL of human NT-4, using the TrkB-transfected cell line, BaF-TrkB-BD. The specific conditions are described in the figure legends.

Additional Applications
Western blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect human NT-4. The detection limit for rhNT-4 is approximately 2 ng/lane and 0.5 ng/lane under non-reducing and reducing conditions, respectively.

Immunohistochemistry - This antibody can be used with appropriate secondary reagents to detect human NT-4. Staining may be done on paraffin-embedded human brain tissues. A working dilution of 10 - 15 µg/mL of the primary antibody is recommended for 5 - 15 µm thick tissue sections fixed on slides. For detection of labeling, it is recommended to employ chromogenic substrates (AEC, DAB, etc.).[1]

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human NT-4. The detection limit for rhNT-4 is approximately 0.08 ng/well.

1. Due to accumulation of autofluorescent pigment lipofucsin in neuronal tissues dissected from non-human primates or humans, the use of fluorescent probes such as FITC or Cy3 is not recommended.

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

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Figure 1

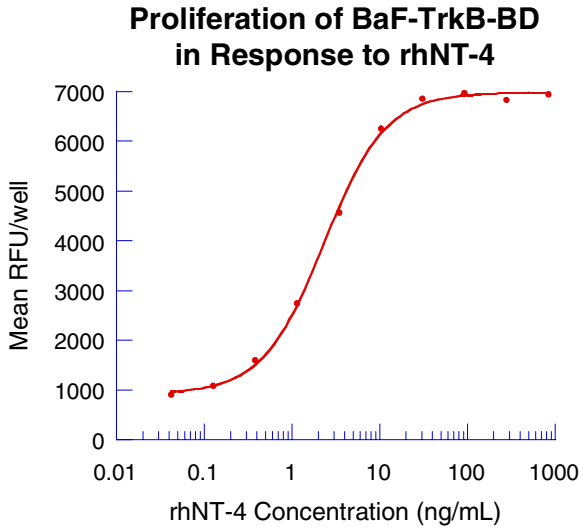

Figure 2

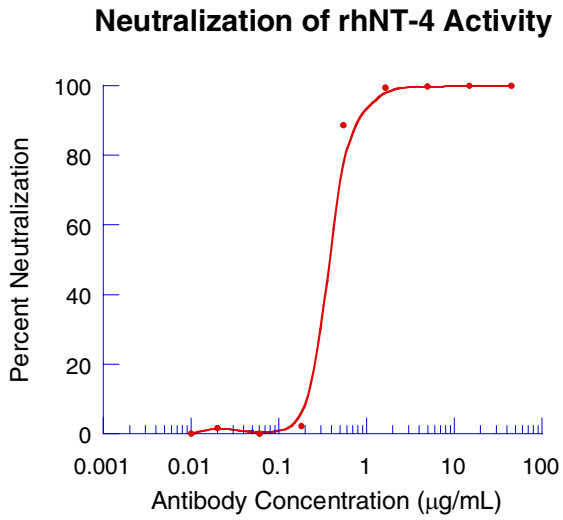

Figure 1
Human NT-4 activity was measured using the TrkB transfected cell line, BaF-TrkB-BD. The ED_{50} for this effect is typically 5 - 15 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the bioactivity of rhNT-4 using BaF-TrkB-BD cells, rhNT-4 was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well microplate. Following this incubation period, BaF-TrkB-BD cells were added. The assay mixure, in a total volume of 100 μL, containing antibody at the concentrations indicated, rhNT-4 at 40 ng/mL and cells at 1 x 10^5 cells/mL, was incubated at 37° C for 3 days in a humidified 5% CO_2 incubator. Resazurin was added during the final 16 - 20 hours of incubation to measure cell growth. The relative fluorescence was then read in a fluorescent plate reader set at Ex. 544/Em. 590. The ND_{50} of the antibody is approximately 1 - 3 μg/mL.