Anti-human FGF-6 Neutralizing Antibody

ORDERING INFORMATION

Catalog Number: AB-238-NA

Lot Number: JK01

Size: 1 mg

Price: $300

Formulation: sterile solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rhFGF-6

Antigen: *E. coli*-derived rhFGF-6

Ig class: goat IgG

Applications: Neutralization of bioactivity
Western blot
ELISA

Preparation

This antibody was produced in goats immunized with purified, *E.coli*-derived, recombinant human fibroblast growth factor 6 (rhFGF-6). 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

< 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 1 mg/mL.

Storage

Lyophilized samples are stable for greater than six months when held at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 4° C for at least 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C for at least 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 rhFGF-6. It will not neutralize the biological activity of rhFGF acidic, rhFGF basic, rhFGF-4, rhFGF-5. In direct ELISA and western blot analysis, this antibody shows less than 17% cross-reactivity with rhFGF-4. Additionally, in direct ELISA, this antibody shows no cross-reactivity with other cytokines tested.[1]

Neutralization of Human FGF-6 Bioactivity

The exact concentration of antibody required to neutralize rhFGF-6 activity is dependent on the biological effect studied, the cell type, and incubation conditions. To provide a guideline, R&D Systems has determined the neutralization dose within a specific cell type for each of its antibodies.

> **Neutralization Dose$_{50}$ (ND$_{50}$)** - that concentration of antibody required to yield one-half maximal inhibition of the cytokine, when that cytokine is present at five times its normal ED$_{50}$ (a concentration of five times the ED$_{50}$ will normally yield 100% activity; figure 1, see page two). The ND$_{50}$ can be used to calculate the amount of antibody needed in a particular application.

The ND$_{50}$ for this lot of anti-human FGF-6 was determined to be approximately 15 - 25 μg/mL in a neutralizing bioassay using FGF-responsive NR6R fibroblasts as target cells. Results of this assay are seen in figure 2 (see page two).

In these experiments rhFGF-6 was pre-incubated with increasing concentrations of antibody for 1 hour at 37° C. Following this preincubation period, the antigen-antibody mixture was added in duplicate to quiescent confluent cultures of NR6R-3T3 cells in DMEM with 2% bovine plasma-derived serum in 96 well microplates. The assay mixture, in a total volume of 100 μL/well, containing rhFGF-6 at a concentration of 1 ng/mL, heparin at a concentration of 1 μg/mL, and antibody at the concentrations indicated, was incubated for 20 hours at 37° C in a 5% CO_2 humidified incubator and pulsed with ^{3}H-thymidine for the final 2 hours. Cells were then detached and harvested onto glass fiber filters and the radioactivity incorporated into DNA counted.

Additional Applications

For direct ELISAs, < 0.62 ng/well of human FGF-6 can be detected using an antibody concentration of 0.5 μg/mL.

For western blot analysis, an antibody concentration of 1 μg/mL will allow visualization of 5 ng/lane of rhFGF-6 under non-reducing and reducing conditions. Since this antibody preparation is a total IgG fraction, complete monospecificity cannot be assumed.

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

Figure 1

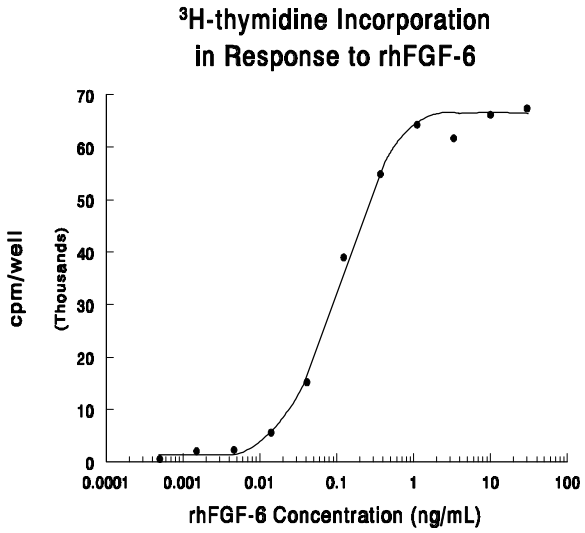
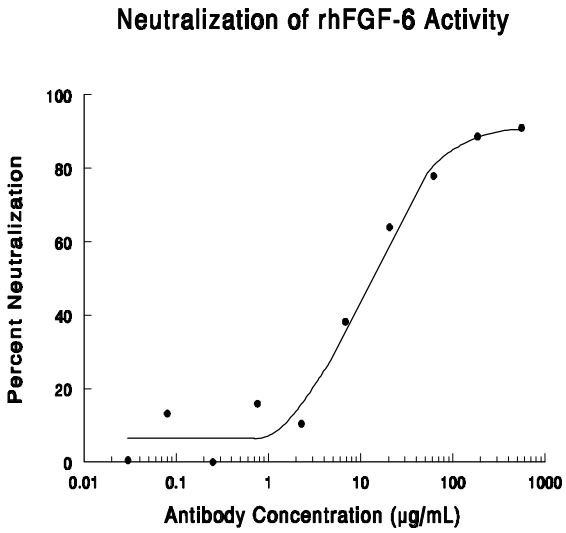


Figure 2



The biological activity of rhFGF-6 was measured in a mitogenic assay using the FGF responsive NR6R fibroblasts (Rizzino, A. *et al*., 1988, Cancer Res. **48**:4266). **Figure 1** shows that cell stimulation, as measured by ^{3}H-thymidine incorporation, is dependent on the concentration of rhFGF-6. The ED_{50} for this effect is typically 0.1 - 0.3 ng/mL of rhFGF-6. Assuming a nominal ED_{50} of 0.2 ng/mL, five times the ED_{50} of rhFGF-6 (1.0 ng/mL) was used to assess the neutralizing activity of this lot of antibody. As seen in figure 2, the ND_{50} for this lot of antibody is approximately 15 - 25 µg/mL.

[1]rhTGF-β1, pTGF-β1.2, pTGF-β2, rcTGF-β3, raTGF-β5, rhLatent TGF-β1, LAP (TGF-β1), rhTGF-β RII, rhTGF-α, rhEGF, rhFGF acidic, rhFGF basic, rhFGF-5, rhPDGF-AA, rhPDGF-BB, rhPDGF-AB, hPDGF, pPDGF, rhb-NGF, rhIL-1α, rmIL-1α, rhIL-1ra, rhIL-1β, rmIL-1β, rhIL-2, rhIL-2 sRα, rhIL-3, rmIL-3, rhIL-4, rmIL-4, rhIL-4 Rα, rhIL-5, rmIL-5, rhIL-5 Rα, rhIL-6, rmIL-6, rhIL-6sR, rhIL-7, rmIL-7, rhIL-8, rhIL-9, rmIL-9, rhIL-10, rmIL-10, rhIL-11, rhIL-12, rhGM-CSF, rmGM-CSF, rhM-CSF, rhLIF, rmLIF, rhMIP-1α, rmMIP-1α, rhMIP-1β, rmMIP-1β, rhTNF-α, rmTNF-α, rhTNF-β, rhsTNF RI, rhsTNF RII, rhRANTES, rhANG, rhOSM, rhSCF, rmSCF, rhSLPI, rhCNTF, rhIFN-γ, rhIGF-I, rhIGF-II, rhGROα, rhEPO, rhMCP-1, rhPTN, rhVEGF, rhHGF