



Anti-human FGF-5 Antibody

ORDERING INFORMATION

Catalog Number: AF-237-NA

Lot Number: AAU03

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rhFGF-5

Immunogen: *E. coli*-derived rhFGF-5

Ig Type: human FGF-5 specific goat IgG

Applications: Neutralization of bioactivity
Western blot
ELISA
Immunohistochemistry

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant human fibroblast growth factor 5 (rhFGF-5). FGF-5 specific IgG was purified by human FGF-5 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 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-5. Based on direct ELISA results, this antibody shows no cross-reactivity with other cytokines tested.¹

Neutralization of Human FGF-5 Bioactivity

The exact concentration of antibody required to neutralize rhFGF-5 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 Dose₅₀ (ND₅₀)** 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 ND₅₀ for this lot of anti-human FGF-5 antibody was determined to be approximately 0.2 - 0.6 µg/mL in the presence of 50 ng/mL of rhFGF-5 and 0.1 µg/mL of heparin, using the FGF-5 responsive NR6R-3T3 fibroblasts as target cells. 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 FGF-5. The detection limit for rhFGF-5 is approximately 0.16 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 FGF-5. The detection limit for rhFGF-5 is approximately 25 ng/lane under both non-reducing and reducing conditions.

For immunohistochemistry, the antibody will detect FGF-5 in paraffin-embedded human tissue sections. The working dilution for 5 - 15 µm thick sections is 10 µg/mL. For chromogenic detection of labeling, it is recommended to use R&D Systems' anti-goat Cell and Tissue Staining kits (CTS Series). It is also recommended to use R&D Systems' antigen retrieval reagents.

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

For immunohistochemistry images, please refer to our website at http://www.rndsystems.com/cyt_cat/ihcprot.html

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1-800-343-7475

Figure 1

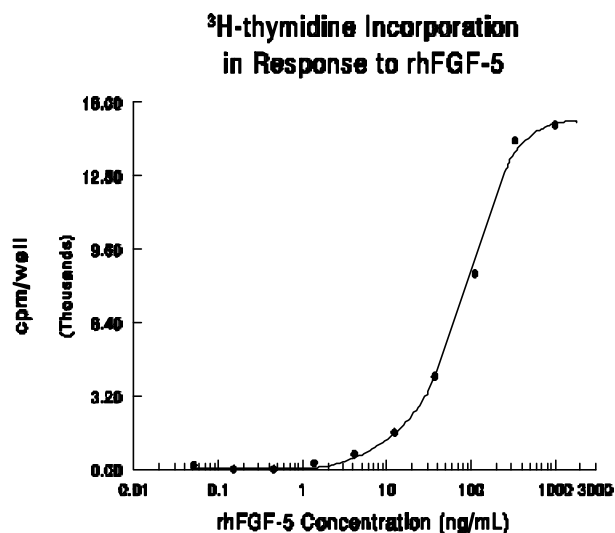


Figure 2

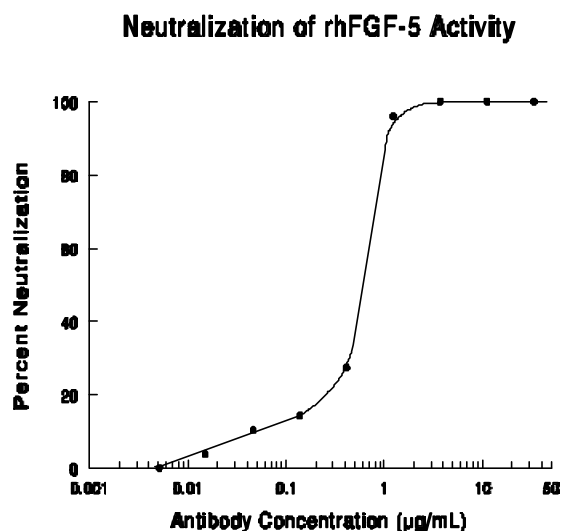


Figure 1

Human FGF-5 stimulates the ^3H -thymidine incorporation by NR6R-3T3 fibroblasts in a dose-dependent manner (Rizzino, A. *et al.*, 1988, *Cancer Res.* **48**:4266 - 4271). The ED_{50} for this effect is typically 50 - 100 ng/mL in the presence of 0.1 $\mu\text{g/mL}$ heparin.

Figure 2

Typical data for anti-hFGF-5 is shown in Figure 2. To measure the ability of the antibody to neutralize the bioactivity of human FGF-5 on NR6R-3T3 fibroblasts, human FGF-5, in the presence of heparin, was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well microtiter plate. Following this preincubation period, the antigen-antibody-heparin mixture was added to quiescent confluent cultures of NR6R-3T3 cells in DMEM with 2% bovine plasma-derived serum. The assay mixture in a total volume of 100 μL , containing antibody at the concentrations indicated, human FGF-5 at 50 ng/mL and 0.1 $\mu\text{g/mL}$ heparin, was incubated at 37° C for 20 hours in a humidified CO_2 incubator. ^3H -thymidine was added during the final 2 hours of incubation. The cells were subsequently detached and harvested onto glass fiber filters and the ^3H -thymidine incorporated into DNA was determined. The ND_{50} of the antibody is approximately 0.2 - 0.6 $\mu\text{g/mL}$.

$^1\text{rhActivin } \beta \text{ A}$, rhActivin RII , rhANG , rhAnnexin V , rhAR , rhB7-1 , rhB7-2 , rmB7-2 , rhBTC , $\text{rh}\beta\text{-NGF}$, $\text{rr}\beta\text{-NGF}$, rhBDNF , rmC10 , rhCD8 , rhCD28 , rrCINC-1 , rhCNTF , rrCNTF , $\text{rhCNTF sR}\alpha$, $\text{rrCNTF sR}\alpha$, rhCTLA-4 , rmCRG-2 , rhEGF , rhENA-78 , rhEotaxin , rmEotaxin , rhEpo , rhEpo R , rhFas , rhFGF acidic , rhFGF basic , rhFGF-4 , rhFGF-6 , rhFGF-7 , rmFGF-8b , rhFGF-9 , $\text{rhFlk2/Flt3 ligand}$, rhFlt-1 R , rhG-CSF , rmG-CSF , $\text{rhG-CSF sR}\alpha$, rmGDF-9 , rhGDNF , rrGDNF , rhGM-CSF , rmGM-CSF , $\text{rhGM-CSF R}\alpha$, $\text{rhGRO}\alpha$, $\text{rhGRO}\beta$, $\text{rhGRO}\gamma$, rhHB-EGF , rhHCC-1 , $\text{rhHRG-}\alpha$, $\text{rhHRG-}\beta$, rhHGF , rhI-309 , $\text{rhIFN-}\gamma$, $\text{rmIFN-}\gamma$, $\text{rrIFN-}\gamma$, rhIGF-I , rhIGF-I bp , rhIGF-I R , rhIGF-II , rhIGIF , $\text{rhIL-1}\alpha$, $\text{rmIL-1}\alpha$, rhIL-1 sRI , rhIL-1 sRII , $\text{rhIL-1}\beta$, $\text{rmIL-1}\beta$, $\text{rrIL-1}\beta$, rhIL-1ra , rmIL-1ra , rhIL-2 , rmIL-2 , rrIL-2 , $\text{rhIL-2 sR}\alpha$, $\text{rhIL-2 sR}\beta$, $\text{rhIL-2 sR}\gamma$, rhIL-3 , rmIL-3 , $\text{rhIL-3 sR}\alpha$, rhIL-4 , rmIL-4 , rrIL-4 , rhIL-4 sR , rhIL-5 , rmIL-5 , $\text{rhIL-5 sR}\alpha$, rhIL-6 , rmIL-6 , rhIL-6 sR , rhIL-7 , rmIL-7 , rhIL-7 R , rhIL-8 , rhIL-9 , rmIL-9 , rhIL-9 sR , rhIL-10 , rmIL-10 , rhIL-10 sR , rmIL-10 sR , rhIL-11 , rmIL-11 , rhIL-12 , rmIL-12 , $\text{rmIL-12 R } \beta$, rhIL-13 , rmIL-13 , rhIL-15 , rhIL-17 , rmIL-17 , rhIP-10 , rmJE , rmKC , rhLIF , rmLIF , rhLIF R , rmLymphotactin , rmMARC , rhM-CSF , rmM-CSF , rhMCP-1 , rmMCP-1 R , rhMCP-2 , rhMCP-3 , rhMidkine , rhMIF , rhMIG , rmMIG , $\text{rhMIP-1}\alpha$, $\text{rmMIP-1}\alpha$, $\text{rhMIP-1}\beta$ (rhACT II), $\text{rmMIP-1}\beta$, $\text{rhMIP-1}\gamma$, rmMIP-2 , $\text{rhMIP-3}\alpha$, $\text{rhMIP-3}\beta$, rhMSP , rhNT-3 , rhNT-4 , rhOB , rmOB , rhOSM , rmOSM , rmPARP , rhPD-ECGF , hPDGF , pPDGF , rhPDGF-AA , rhPDGF-AB , rhPDGF-BB , rrPDGF-BB , $\text{rhPDGF R}\alpha$, rhPIGF , rhPTN , rhRANTES , rmRANTES , rhSCF , rmSCF , rhSCF R , $\text{rhSDF-1}\alpha$, $\text{rhSDF-1}\beta$, rhsgp130 , rmSHH , rhSLPI , rhTarc , rmTECK , hTfR , $\text{rhTGF-}\alpha$, $\text{rhTGF-}\beta 1$, $\text{rhTGF-}\beta 2$, $\text{rhTGF-}\beta 3$, $\text{raTGF-}\beta 5$, $\text{rhLAP (TGF-}\beta 1)$, $\text{rhLatent TGF-}\beta 1$, $\text{rhTGF-}\beta \text{ sRII}$, $\text{rhTGF-}\beta \text{ sRIII}$, $\text{rhTNF-}\alpha$, $\text{rmTNF-}\alpha$, $\text{rrTNF-}\alpha$, $\text{rhTNF-}\beta$, rhsTNF RI , rmsTNF RI , rhsTNF RII , rmsTNF RII , rhTpo , rmTpo , rhVEGF , rmVEGF , rhVEGF/PIGF