



Anti-PDGF-BB Antibody

ORDERING INFORMATION

Catalog Number: AF-220-NA

Lot Number: ABO04

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: PDGF-BB, hPDGF-AB

Immunogen: *E. coli*-derived rhPDGF-BB

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation

Produced in goats immunized with purified, *E. coli*-derived, recombinant human platelet-derived growth factor BB (rhPDGF-BB; Ser82 - Thr190; Accession # P01127). PDGF-BB specific IgG was purified by human PDGF-BB 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.**

Specificity

This antibody has been selected for its ability to neutralize the biological activity of rhPDGF-BB. It will also neutralize the biological activity of natural human PDGF-AB, rhPDGF-AB, rat PDGF-BB and porcine PDGF-BB. This antibody will neutralize rhPDGF-AA, at a 50 - 100 fold higher IgG concentration. Based on Western blot results (non-reducing conditions), this antibody shows less than 10% cross-reactivity with rhPDGF-AA.

Neutralization of Human PDGF-BB bioactivity

The exact concentration of antibody required to neutralize PDGF-BB 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-PDGF-BB antibody was determined to be approximately 0.1 - 0.5 µg/mL in the presence of 10 ng/mL of rhPDGF-BB using PDGF-responsive NR6R-3T3 fibroblasts as target cells. The specific conditions are described in the figure legends.

Additional Applications

Western blot - This antibody can be used at 0.1 - 0.2 µg/mL with the appropriate secondary reagents to detect PDGF-BB and PDGF-AB. The detection limit for rhPDGF-BB and PDGF-AB is approximately 0.5 ng/lane and 5 ng/lane under non-reducing and reducing conditions, respectively.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect PDGF-BB and PDGF-AB. The detection limit for PDGF-BB and PDGF-AB is approximately 0.3 ng/well.

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

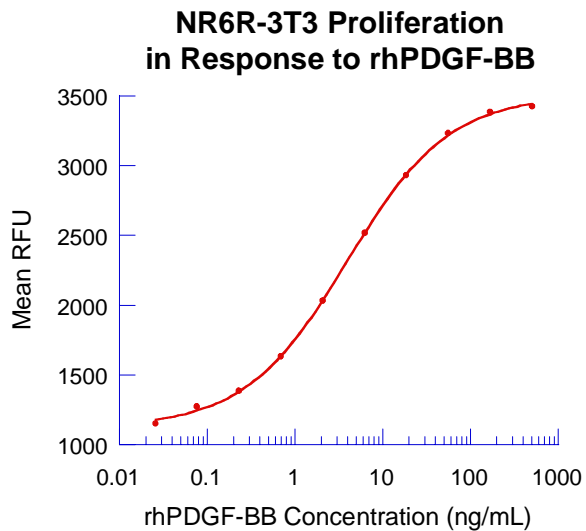


Figure 2

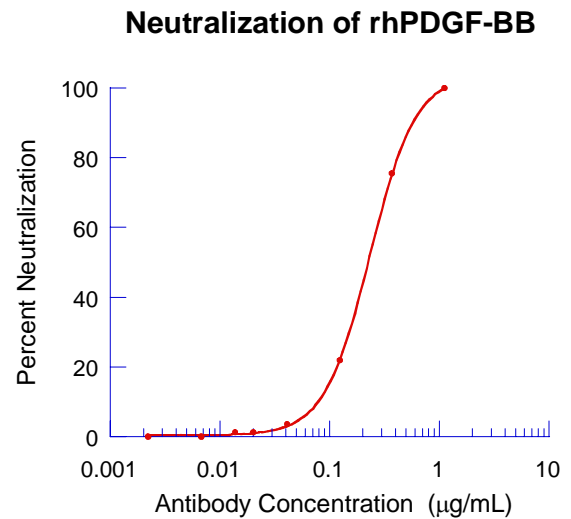


Figure 1

rhPDGF-BB stimulates the proliferation of NR6R-3T3 fibroblasts in a dose-dependent manner (Raines, E.W. *et al.*, 1985, *Methods in Enzymology* **109**:749 - 773). The ED₅₀ for this effect is typically 1.5 - 6 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize the bioactivity of rhPDGF-BB on NR6R-3T3 cells, rhPDGF-BB was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well plate. Following this preincubation period, NR6R-3T3 cells were added. The assay mixture in a total volume of 100 µL, containing antibody at the concentrations indicated, rhPDGF-BB at 10 ng/mL, and cells at 5 x 10⁴ cells/mL, was incubated at 37° C for 72 hours in a humidified CO₂ incubator and pulsed with Resazurin (R&D Systems, Catalog # AR002) for the final 16 - 20 hours. The fluorescence was then read in a microplate plate reader set at 544/590 nm. The ND₅₀ of the antibody is approximately 0.1 - 0.5 µg/mL.