

ORDERING INFORMATION

Catalog Number: AF1143

Lot Number: GNY01

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human PD-ECGF

Immunogen: Sf 21-derived rhPD-ECGF

Ig Type: goat IgG

Applications: Neutralization of bioactivity Western blot Direct ELISA Immunohistochemistry

Anti-human PD-ECGF Antibody

Preparation

Produced in goats immunized with purified, insect cell line *Sf* 21-derived, recombinant human platelet-derived endothelial cell growth factor (rhPD-ECGF). Human PD-ECGF specific IgG was purified by human PD-ECGF affinity chromatography.

Formulation

Lyophilized from a 0.2 μm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Endotoxin Level

< 0.01 EU per 1 μ g 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 was selected for its ability to neutralize rhPD-ECGF bioactivity.

Neutralization of Human PD-ECGF Bioactivity

The exact concentration of antibody required to neutralize rhPD-ECGF 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 ND₅₀ for this lot of anti-human PD-ECGF antibody was determined to be approximately 0.5 - 2 μ g/mL in the presence of 150 ng/mL of rhPD-ECGF, using PD-ECGF responsive HUVE cells. The specific conditions are described in the figure legends.

Additional Applications

Western blot - This antibody can be used at 0.1 - 0.2 μ g/mL with the appropriate secondary reagents to detect human PD-ECGF. The detection limit for rhPD-ECGF is approximately 5 ng/lane under non-reducing and reducing conditions.

Direct ELISA - This antibody can be used at 0.5 - 1.0 μ g/mL with the appropriate secondary reagents to detect human PD-ECGF. The detection limit for rhPD-ECGF is approximately 0.2 ng/well.

Immunohistochemistry - This antibody will detect PD-ECGF in cells and tissues. The working dilution is 2 - 5 μ g/mL. For chromogenic detection of labeling, use R&D Systems' Cell and Tissue Staining Kits (CTS Series).

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

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.



Figure 2

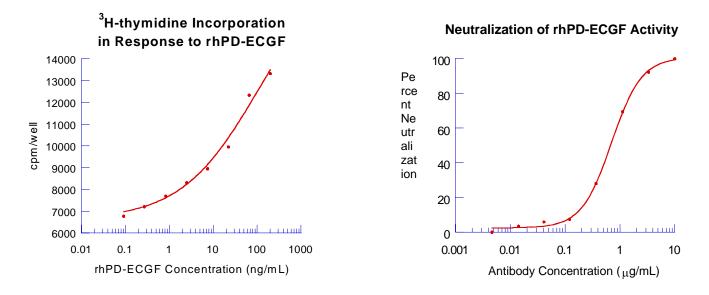


Figure 1

Human PD-ECGF stimulates the ³H-thymidine incorporation by human umbilical vein endothelial cells (Usuki, K., *et al.*, 1990, Cell Regulation 1:577). The ED₅₀ for this effect is typically 20 - 40 ng/mL.

Figure 2

To measure the ability of the antibody to neutralize rhPD-ECGF response, rhPD-ECGF was added to a 96 well plate containing various concentrations of antibody and preincubated for 1 hour at room temperature. Following this preincubation, HUVE cells were added. The assay mixture in a total volume of 100 μ L, containing antibody at the concentrations indicated, rhPD-ECGF at 150 ng/mL and cells at 1 x 10⁵ cells/mL, was incubated at 37° C for 72 hours in a humidified CO₂ incubator. ³H-thymidine was added during the final 20 hours of incubation. The cells were subsequently detached and harvested onto glass fiber filters and the ³H-thymidine incorporated into DNA was determined. The ND₅₀ of the antibody under these conditions is approximately 0.5 - 2 μ g/mL.