Anti-zebrafish VEGF Antibody

ORDERING INFORMATION

Catalog Number: AF1247

Lot Number: UCD01

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: zebrafish VEGF

Immunogen: *Sf* 21-derived rzfVEGF$_{165}$

Ig Type: goat IgG

Applications: Neutralization of bioactivity
Western blot
Direct ELISA

Preparation
Produced in goats immunized with purified, *Sf* 21-derived, recombinant zebrafish Vascular Endothelial Growth Factor (rzfVEGF$_{165}$). Zebrafish VEGF specific IgG was purified by zebrafish VEGF 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 0.5 mL of PBS is used, the antibody concentration will be 0.2 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 zebrafish VEGF bioactivity.

Neutralization of Zebrafish VEGF Bioactivity
The exact concentration of antibody required to neutralize zebrafish VEGF 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.

The ND$_{50}$ for this lot of anti-zebrafish VEGF antibody was determined to be approximately 0.3 - 0.6 μg/mL in the presence of 50 ng/mL of rzfVEGF, using the HUVE cell line. The specific conditions are described in the figure legends.

Additional Applications
Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect zebrafish VEGF. The detection limit for rzfVEGF is approximately 0.2 ng/well.

Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect zebrafish VEGF. The detection limit for rzfVEGF is approximately 1 ng/lane and 5 ng/lane under non-reducing and reducing conditions, respectively. In this format, this antibody shows less than 2% cross-reactivity with rhVEGF$_{165}$, rmVEGF$_{164}$ and rrVEGF$_{164}$ under non-reducing conditions.

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

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

Figure 1

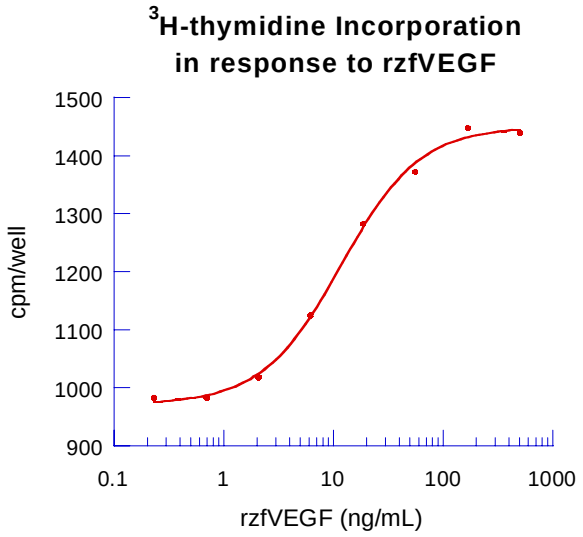

Figure 2

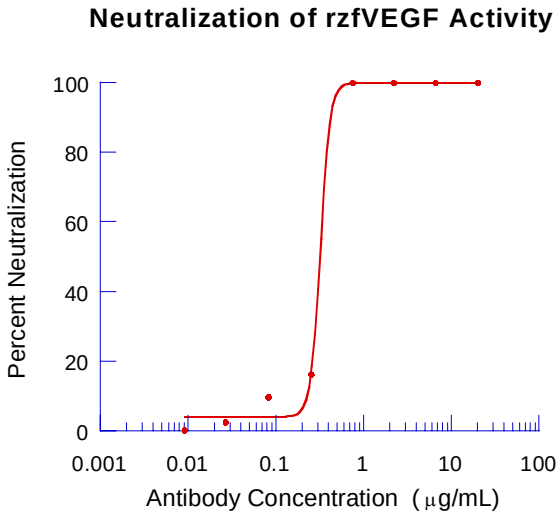

Figure 1
Zebrafish VEGF stimulates the ^{3}H-thymidine incorporation by human umbilical vein endothelial cells in a dose-dependent manner. The ED_{50} for this effect is typically 20 - 60 ng/mL.

Figure 2
To measure the ability of the antibody to neutralize the bioactivity of rzfVEGF on human umbilical vein endothelial cells, rzfVEGF was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96-well microplate. Following this preincubation period, HUVE cells were added. The assay mixture, in a total volume of 100 µL, containing antibody at the concentration indicated, rzfVEGF at 80 ng/mL and cells at 5 x 10^4 cells/mL, was incubated at 37° C for 3 days in a humidified CO_2 incubator. ^{3}H-thymidine was added during the final 20 hours of incubation. The cells were subsequently harvested onto glass fiber filter and the ^{3}H-thymidine incorporated into DNA was determined. The ND_{50} of this antibody is approximately 0.3 - 0.6 µg/mL.