



# Anti-mouse GDF-8 propeptide Antibody

## ORDERING INFORMATION

**Catalog Number:** AF1539

**Lot Number:** UTG01

**Size:** 100 µg

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

**Storage:** -20° C

**Reconstitution:** sterile PBS

**Specificity:** mouse GDF-8 propeptide

**Immunogen:** NS0-derived rmGDF-8 propeptide

**Ig Type:** sheep IgG

**Applications:** Neutralization of bioactivity  
Western blot  
ELISA

## Preparation

Produced in sheep immunized with purified, NS0-derived, recombinant mouse Growth Differentiation Factor 8 propeptide (rmGDF-8 propeptide). Mouse GDF-8 propeptide specific IgG was purified by mouse GDF-8 propeptide affinity chromatography. GDF-8, also known as Myostatin, belongs to the TGF-β superfamily. It is synthesized as an inactive proprotein with the N-terminal pro-region and the C-terminal mature bioactive region. The GDF-8 proregion is capable of associating with active GDF-8 with high affinity and is a potent GDF-8 antagonist. The pro region of human and mouse GDF-8 shares 96% amino acid sequence identity.

## 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 mouse GDF-8 propeptide bioactivity.

## Neutralization of Mouse GDF-8 propeptide Bioactivity

The exact concentration of antibody required to neutralize mouse GDF-8 propeptide 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<sub>50</sub> (ND<sub>50</sub>)** 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<sub>50</sub> for this lot of anti-mouse GDF-8 propeptide antibody was determined to be approximately 10 - 30 µg/mL in the presence of 800 ng/mL of rmGDF-8 propeptide, and 40 ng/mL of rmGDF-8, using K562 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 mouse GDF-8 propeptide. The detection limit for rmGDF-8 propeptide is approximately 2 ng/lane under non-reducing and reducing conditions.

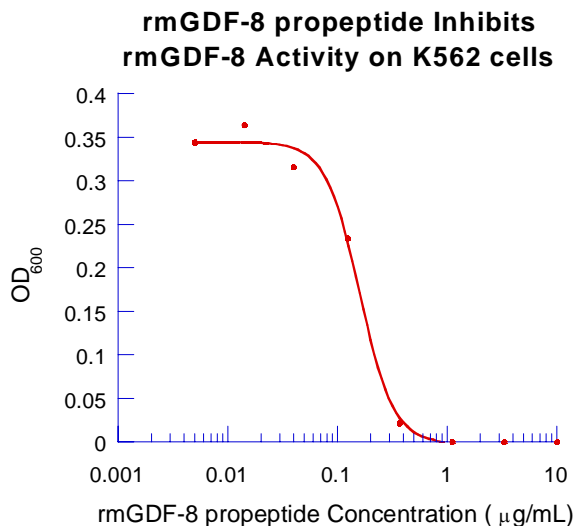
**Direct ELISA** - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect mouse GDF-8 propeptide. The detection limit for rmGDF-8 propeptide is approximately 0.3 ng/well. In this format, this antibody shows less than 10% cross-reactivity with mature rmGDF-8 and less than 5% cross-reactivity with mature rmGDF-1, -3, -5, -6, -7, -9, and rhGDF-11.

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

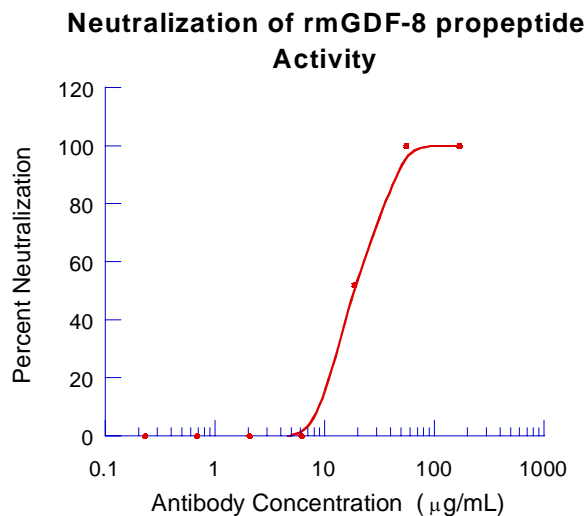
FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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

**Figure 1**



**Figure 2**



**Figure 1**

Mouse GDF-8 propeptide inhibits mouse GDF-8 induced hemoglobin expression in K562 cells in a dose dependent manner. The ED<sub>50</sub> for this effect is typically 0.05 - 0.25 µg/mL.

**Figure 2**

To measure the ability of the antibody to neutralize the bioactivity of mouse GDF-8 propeptide, it was added at a constant concentration and incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well plate. Mouse GDF-8 was then added to each well at a constant concentration and the plate was incubated for an additional hour at 37° C. Following this preincubation period, K562 cells were added. The assay mixture in a total volume of 200 µL per well, containing antibody at the concentrations indicated, mouse GDF-8 propeptide at 800 ng/mL, rmGDF-8 at 40 ng/mL, and cells at 2.5 x 10<sup>4</sup> cells/mL, was incubated at 37° C for 4 days in a humidified CO<sub>2</sub> incubator. At the end of incubation, the hemoglobin level in cell lysate was measured by its pseudoperoxidase activity. This antibody will neutralize 100% of rmGDF-8 propeptide bioactivity on K562 cells. The ND<sub>50</sub> for this effect is typically 10 - 30 µg/mL.