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# **ProductInformation**

# Anti-Insulin-Like Growth Factor II (IGF-II)

Produced in Goat, Affinity Isolated Antibody

Product Number I 2282

#### **Product Description**

Anti-Mouse Insulin-like Growth Factor-II (IGF-II) is produced in goat using recombinant mouse insulin-like growth factor-II (IGF-II)<sup>1</sup> expressed in *Escherichia coli*, as the immunogen. The antibody is purified by IGF-II affinity chromatography.

Anti-Mouse Insulin-like Growth Factor-II (IGF-II) recognizes recombinant mouse IGF-II by various immunochemical techniques including neutralization, immunoblotting, and ELISA. Based on ELISA, this antibody shows approximately 25% cross-reactivity with recombinant human IGF-II.

Insulin-like growth factor II (also known as multiplication stimulating activity or MSA) and insulin-like growth factor I (IGF-I) belong to the family of insulin-like growth factors which are structurally homologous to proinsulin. Mature IGF-I and IGF-II are highly conserved and share approximately 70% amino acid sequence identity. Mouse IGF-II, a 67 amino acid protein, has a predicted molecular mass of approximately 7.4 kDa. Mouse and human IGF-II share 91% sequence identity.

Insulin-like growth factor II has autocrine, paracrine, and endocrine functions. It is a potent mitogenic growth factor that mediates growth-promoting activities in embryonic development. IGF-II binds the IGF-II receptor with high affinity.

IGF-I and IGF II are expressed in many tissues and cell types. IGF-II is mitogenic for a variety of cultured cells including human or chicken fibroblasts, mouse 3T3 cells, normal rat kidney cells, and MCF-7 human breast carcinoma cells.<sup>2</sup>

## Reagent

The antibody is supplied as ~100  $\mu g$  of antiserum lyophilized from a 0.2  $\mu m$  filtered solution in phosphate buffered saline with 5% trehalose.

### **Preparation Instructions**

To one vial of lyophilized powder, add 1 ml of sterile phosphate-buffered saline containing 0.1% human serum albumin or bovine serum albumin to produce a 0.1 mg/mL stock solution of antibody.

#### Storage/Stability

Prior to reconstitution, store at -20 °C. Reconstituted antibody may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Avoid repeated freezing and thawing. Do not store in a "frost-free" freezer.

#### **Product Profile**

Anti-Mouse Insulin-Like Growth Factor II has the ability to neutralize the bioactivty of recombinant mouse IGF-II on MCF-7 cells. Recombinant mouse IGF-II (30 ng/ml) is added to the wells of a 96 well plate containing various concentrations (0.01-100 µg/ml) of the antibody and preincubated for 1 hour at 37 °C. Following this preincubation, MCF-7 cells (5 x 10<sup>4</sup> cells/ml) are added to the wells. The assay mixture in a total volume of 100 µl, containing antibody at concentrations of 0.01-100 µg/ml, recombinant mouse IGF-II at 30 ng/ml, and cells at 5 x 10<sup>4</sup> cells/ml, is incubated at 37 °C for 72 hours in a humidified CO<sub>2</sub> incubator. The mixture is pulsed with <sup>3</sup>H-thymidine during the final 24 hours. The cells are detached and harvested onto glass fiber filters, and the <sup>3</sup>H-thymidine incorporated into the DNA is measured.3

The exact concentration of antibody required to neutralize recombinant mouse IGF-II activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity.

The Neutralization  $Dose_{50}$  (ND<sub>50</sub>) for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the IGF-II activity on a responsive cell line, when IGF-II is present at a concentration just high enough to elicit a maximum response.

For immunoblotting, a working antibody concentration of 0.1 to 0.2 µg/mL is recommended. The detection limit

for recombinant mouse IGF-II is approximately 20 ng/lane and 0.5 ng/lane under non-reducing and reducing conditions, respectively.

For ELISAs, a working antibody concentration of 0.5 to 1.0 µg/mL is recommended. The detection limit for recombinant mouse IGF-II is approximately 0.5 ng/well.

#### References

- 1. Stempien, M., et al., DNA, 5, 357 (1986).
- Zumstein, P., et al., J. Biol. Chem., 262, 11252 (1987).
- Karey, K.P., et al., Cancer Research, 48, 4083 (1988).

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