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# **Product Information**

# Anti-Insulin-Like Growth Factor-I produced in goat, affinity isolated antibody

Catalog Number 18773

## **Product Description**

Anti-Insulin-Like Growth Factor-I (IGF-I) was produced in goat using recombinant human insulin-like growth factor-I (IGF-I), expressed in *Escherichia coli*, as the immunogen. The product is purified by IGF-I affinity chromatography.

Anti-Insulin-Like Growth Factor-I recognizes recombinant human IGF-I by various immunochemical techniques including neutralization and immunoblotting, The antibody does not neutralize the biological activity of recombinant human IGF-II.

Insulin-Like Growth Factor-I is a single chain polypeptide of 70 amino acid residues cross-linked by three disulfide bridges.<sup>1</sup> It is mitogenic for a variety of cells including fibroblasts, osteoblasts, smooth muscle cells, fetal brain cells, neuroglial cells, and erythroid progenitor cells.<sup>2</sup>

#### Reagent

Supplied as 250  $\mu g$  of antiserum lyophilized from a 0.2  $\mu m$  filtered solution of phosphate buffered saline with 5% trehalose.

## **Preparation Instructions**

To one vial of lyophilized powder, add 1 ml of  $0.2 \mu m$  filtered phosphate buffered saline to produce a  $0.25 \mu m$  mg/ml stock solution of antibody.

#### Storage/Stability

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

#### **Product Profile**

Neutralization: Anti-IGF-I has the ability to neutralize the biological activity of recombinant human IGF-I on MCF-7 cells. Recombinant human IGF-I is added to various concentrations of the antibody for 1 hour at 37 °C in a 96 well plate. Following this pre-incubation, the antigen-antibody mixture is added to MCF-7 cells.

The assay mixture in a total volume of 100  $\mu$ L, containing antibody at concentrations of 0.01-200  $\mu$ g/ml, recombinant human IGF-I at 6 ng/ml, and cells at  $5x10^4$  cells/ml, is incubated at 37 °C for 72 hours in a humidified  $CO_2$  incubator. The mixture is pulsed with  $^3$ H-thymidine during the final 24 hours. The cells are detached and harvested onto glass fiber filters, and the  $^3$ H-thymidine incorporated into the DNA is measured.

The Neutralization  $\mathsf{Dose}_{50}$  (ND<sub>50</sub>) is 3-12  $\mu \mathsf{g/ml}$  in the presence of 6 ng/ml of recombinant human IGF-I using MCF-7 cells.

The  $ND_{50}$  of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of bioactivity of recombinant human IGF-I, when IGF-I is present at a concentration just high enough to elicit a maximum response.

**Note**: The exact concentration of antibody required to neutralize recombinant human IGF-I activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working antibody concentration of  $0.1\text{-}0.2~\mu\text{g/ml}$  is recommended. The detection limit of recombinant human IGF-I is ~20 ng/lane and 2 ng/lane under non-reducing and reducing conditions, respectively.

**Note**: In order to obtain the best results in various techniques and preparations, we recommend determining the optimal working dilutions by titration.

Endotoxin level determined by the LAL (Limulus amebocyte lysate) method found less than 0.1 EU per 1  $\mu g$  of antibody.

#### References

- 1. Rinderknecht, E., et al., *J. Biol. Chem.*, **256**, 2769 (1978).
- 2. Zumstein, P., et al., *J. Biol. Chem.*, **262**, 11252 (1987).

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