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ProductInformation

Anti-Insulin-like Growth Factor Binding Protein-4
produced in goat, affinity isolated antibody

Product Number **I7026**

Product Description

Anti-Insulin-like Growth Factor Binding Protein-4 (IGFBP-4) is developed in goat using a purified recombinant human insulin-like growth factor binding protein-4, expressed in mouse NSO cells, as immunogen. Affinity isolated antibody is obtained from goat anti-IGFBP-4 antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Insulin-like Growth Factor Binding Protein-4 recognizes recombinant human IGFBP-4 by various immunochemical techniques including neutralization, immunoblotting, and ELISA. Based on ELISA and immunoblotting, this antibody shows no cross-reactivity with recombinant human IGFBP-1, recombinant human IGFBP-2, and recombinant human IGFBP-3, recombinant human IGFBP-5, and recombinant human IGFBP-6.

Insulin-like growth factor binding protein-4 is produced from a DNA sequence encoding the human IGFBP-4 protein.[1] Mature human IGFBP-4 has a calculated molecular mass of ~26 kDa. Due to glycosylation, the recombinant protein migrates as a 32 kDa and 25 kDa protein under reducing and non-reducing conditions, respectively. Human IGFBP-4 has a potential N-linked glycosylation site and shares approximately 90% amino acid sequence identity with both mouse and rat IGFBP-4.

IGFBP-4 is a member of the superfamily of insulin-like growth factor (IGF) binding proteins which include six high-affinity IGF binding proteins and at least four low-affinity binding proteins referred to as IGFBP-related proteins (IGFBP-rP). The IGFBP members are cysteine-rich proteins with conserved cysteine residues clustered in the amino-terminal and the carboxy-terminal regions of the molecule. IGFBP-4 was isolated from human plasma based on its ability to bind immobilized IGF-1.

IGFBPs hold a central position in IGF ligand-receptor interactions through influences on both the bioavailability and distribution of IGFs in the extracellular environment.[2] IGFBPs will either inhibit or enhance the biological activities of IGF or act in an IGF-independent manner. Post-translational modification of IGFBPs, including phosphorylation and proteolysis, will modify the affinities of the binding proteins for IGF and may indirectly regulate IGF actions. IGFBP-4 functions as an inhibitor of IGF action and its main function may be to protect cells from overstimulation by IGFs or to allow activation of alternate transmembrane signaling pathways that are inhibited by IGF exposure.[3]

IGFBP-4 is expressed in multiple tissues including adrenal, testis, spleen, heart, lung, kidney, liver, stomach, hypothalamus, and brain cortex. High expression levels are found in the non-paranchymal cells of the liver.[4] IGFBP-4 is the predominant IGFBP expressed by human osteoblast-like cells and is also expressed in skin fibroblasts.[5]

Reagent

Supplied lyophilized from a 0.2 µm filtered solution of phosphate buffered saline containing 5% trehalose.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of sterile phosphate buffered saline (PBS) to produce a 0.1 mg/mL stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product 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 frost-free freezer.

Product Profile
Anti-IGFBP-4 has the ability to neutralize the biological activity of recombinant human IGFBP-4 on human breast cancer (MCF-7) cells in the presence of recombinant human IGF-II. Recombinant human IGFBP-4 is added to various concentrations of the antibody for 30 minutes at 37 °C in a 96 well plate. Following this pre-incubation, recombinant human IGF-II is added to the mixture and incubated for an additional 30 minutes at 37 °C. MCF-7 cells are then added to the mixture. The assay mixture, in a total volume of 100 µL containing antibody at concentrations of 0.5 µg/mL to 150 µg/mL, recombinant human IGFBP-4 at 0.3 µg/mL, recombinant human IGF-II at 14 ng/mL, and cells at 5 x 10^4 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.[6]

The Neutralization $Dose_{50}$ (ND_{50}) for Anti-IGFBP-4 is 10-30 µg/mL in the presence of 0.3 µg/mL of recombinant human IGFBP-4 and 14 ng/mL of recombinant human IGF-II using the human breast cancer cell line MCF-7 cells.

The ND_{50} is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response.

The exact concentration of antibody required to neutralize human IGFBP-4 activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working concentration of 0.1-0.2 µg/mL antibody is recommended. The detection limit for recombinant human IGFBP-4 is ~1 ng/lane under non-reducing and reducing conditions.

ELISA: a working concentration of 0.5-1 µg/mL antibody is recommended. The detection limit for recombinant human IGFBP-4 is ~0.16 ng/well.

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

Endotoxin level is <10 ng/mg antibody as determined by the LAL (Limulus amebocyte lysate) method.

References

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