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ProductInformation

Anti-Fibroblast Growth Factor-18
Developed in Goat
Affinity Isolated Antibody

Product Number **F 3053**

Product Description

Anti-Human Fibroblast Growth Factor-18 (FGF-18) is developed in goat using a purified recombinant human fibroblastic growth factor 18[1, 2] expressed in *Escherichia coli* as immunogen. Affinity isolated antigen specific antibody is obtained from goat anti-FGF-18 antiserum by immuno-specific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to the peptide.

Anti-Fibroblast Growth Factor-18 recognizes recombinant human FGF-18 by various immunochemical techniques including neutralization, immunoblotting, and ELISA. Based on ELISA, this antibody shows approximately 6% cross-reactivity with human FGF-17, human FGF-8b, mouse FGF-8b, and mouse FGF-8c. There is no cross-reactivity with human FGF acidic, human FGF basic, human FGF-4, human FGF-6, human FGF-7 human FGF-9, and human FGF-10.

Fibroblast growth factors (FGFs) are members of a large family of structurally related polypeptides (17 kDa to 38 kDa) that exert biological activities toward cells of mesenchymal, neuronal, and epithelial origin.[3, 4] All members of the FGF superfamily have two conserved cysteine residues and a conserved 120 amino acid core region that contains six identical, interspersed amino acids.[5-7] All FGFs share 30% to 50% amino acid sequence identity. FGFs are involved in normal development, wound healing and repair, angiogenesis, and a variety of neurotrophic activities. They are also involved in hematopoiesis as well as in tissue remodeling and maintenance. FGFs are potent physiological regulators of growth and differentiation for a variety of cells of mesodermal, ectodermal, and endodermal origin. They have been implicated in pathological conditions such as tumorigenesis and metastasis. To date, the FGF family consists of 23 members designated FGF-1 through FGF-23.[7]

Four distinct tyrosine kinase FGF receptors (FGFRs) from four separate genes have been identified: FGFR-1 (flg, cek-1), FGFR-2 (bek, cek-3), FGFR-3 (cek-2), and FGFR-4.[8, 9, 10] These high affinity cell surface FGF receptors have an extracellular region containing three immunoglobulin-like domains, a transmembrane region, and a cytosolic tyrosine kinase domain activated by ligand binding. Multiple additional variants (isoforms) arising from alternative splicing have also been reported.[9] Ligand binding specificity, signal transduction, and membrane attachment may be modified by alternative splicings.

Fibroblast Growth Factor 18 (FGF-18), a secreted, heparin binding glycoprotein, was originally isolated from rat embryos using homology-based PCR. It is most similar to FGF-8 and FGF-17 sharing 52% amino acid identity. Because of this homology, the gene may code for alternate splice forms. Recombinant human FGF-18 has a predicted molecular mass of approximately 21 kDa. Human FGF-18 shares 99% amino acid identity with both mouse and rat FGF-18.[1, 2]

FGF-18, a pleiotropic growth factor, stimulates proliferation in a number of tissues, most notably the liver and small intestine. It is expressed in both fetal and adult tissues and appears to be involved in lung physiology since it is present in both the adult and fetal respiratory systems.[1] FGF-18 may be the endogenous ligand for FGF R3.[11]

Reagent

Anti-Fibroblast Growth Factor-18 is supplied as 100 µg of antiserum lyophilized from a 0.2 µm filtered solution of phosphate buffered saline (PBS).

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 at least 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-Fibroblast Growth Factor-18 has the ability to neutralize the biological activity of human FGF-18 on NR6R-3T3 fibroblasts. Human FGF-18, in the presence of heparin, 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 quiescent confluent cultures of NR6R-3T3 cells in DMEM with 2% bovine plasma-derived serum. The assay mixture in a total volume of 100 µl, containing antibody at concentrations of 0.1 µg/ml to 100 µg/ml, human FGF-18 at 20 ng/ml, heparin at 0.1 µg/ml, and the confluent cell layer is incubated at 37 °C for 18-20 hours in a humidified CO_2 incubator. The mixture is pulsed with ^{3}H-thymidine during the final 2 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-human FGF-18 is approximately 4-12 µg/ml in the presence of 20 ng/ml of recombinant human FGF-18 and 0.1 µg/ml of heparin using the NR6R-3T3 cell line.

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 FGF-18 activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

For immunoblotting, a working antibody concentration of 0.1-0.2 µg/ml is recommended. The detection limit for recombinant human FGF-18 is approximately 5 ng/lane under non-reducing and reducing conditions.

For ELISAs, a working antibody concentration of 0.5-1.0 µg/ml is recommended. The detection limit for recombinant human FGF-18 is approximately 0.6 ng/well.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining the 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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