3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Monoclonal Anti-Fibroblast Growth Factor-8
Clone 47109.111
produced in mouse, purified immunoglobulin

Catalog Number **F3178**

Product Description

Monoclonal Anti-Fibroblast Growth Factor-8 (FGF-8) (mouse IgG1) is produced from a mouse hybridoma elicited from a mouse immunized with purified recombinant mouse fibroblast growth factor 8b[1] expressed in *Escherichia coli* as immunogen. The antibody is purified from the IgG fraction of ascities fluid using protein G.

Monoclonal Anti-Fibroblast Growth Factor-8 has the ability to neutralize the biological activity of FGF-8b and FGF-8c. The antibody recognizes human and mouse FGF-8b and FGF-8c. Various immunochemical techniques may be used including ELISA, immunoblotting, immunohistochemisty, and neutralization. Based on immunoblotting and ELISA, the antibody shows less than 2% cross-reactivity with recombinant human (rh) FGF-5, rhFGF-7, and rhFGF-9.

Fibroblast growth factors (FGFs) are members of a large family of structurally related polypeptides (17-38 kDa) that exert biological activities toward cells of mesenchymal, neuronal, and epithelial origin.[2, 3] 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.[4-6] 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. The FGF family consists of 23 members designated FGF-1 through FGF-23.[6] 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.[7-9] 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.[8] Ligand binding specificity, signal transduction, and membrane attachment may be modified by alternative splicings.

Fibroblast Growth Factor 8 was originally identified as an androgen-dependent growth factor of mouse mammary carcinoma cells.[10] The primary transcript of mouse FGF-8 is alternatively spliced to generate at least eight secreted isoforms that differ at their amino terminus. The differences between the isoforms exist in the number of potential N-linked glycosylation sites.[1, 11] In mouse, the eight isoforms are labeled as 8a through 8h. Human FGF-8 is limited to only four isoforms. Only isoforms 8a, 8b, 8e, and 8f are synthesized in humans. Mouse and human 8a and 8b isoforms are 100% identical, while the 8e and 8f isoforms are 98% identical.[1, 11] The FGF-8 isoforms differentially activate the various alternatively spliced forms of the FGF receptors 1-3, and FGF receptor 4. The isoform FGF-8c activates FGF R3c and FGF R4.[12]

Expression of FGF-8 is restricted to embryonic days 9-13 in the mouse. During mouse development, the expression pattern suggests a role for FGF-8 in ectodermal differentiation of the post-gastrulation mouse embryo that includes a role in outgrowth and patterning of the face, limbs, and central nervous system of the vertebrate. In the adult, FGF-8 is found in prespermatogonia and antral follicles of the ovary.[13]

The gene for mouse FGF-8 has been mapped in mouse to chromosome 19 and in human to chromosome 10.

Reagent

Supplied as 500 µg of antiserum lyophilized from a 0.2 µm filtered solution of phosphate buffered saline and 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 to produce a 0.5 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-Fibroblast Growth Factor-8 has the ability to neutralize the biological activity of FGF-8c on NR6R-3T3 fibroblasts in the presence of 0.1 μg/mL of heparin with either 10 ng/mL of recombinant human FGF-8b or 125 ng/mL FGF-8c. Mouse FGF-8c, 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-heparin 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 (0.2-100 μg/mL), mouse FGF-8c (125 ng/mL), heparin (0.1 μg/mL), and the confluent NR6R-3T3 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.[14, 15]

Note: At saturating antibody concentrations, only 60-80% of the FGF-8b bioactivity can be inhibited. The exact concentration of antibody required to neutralize FGF-8 activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied. 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.

Immunoblotting: a working concentration of 1-2 μg/mL is recommended to detect FGF-8 and FGF-8c. The detection limit for FGF-8b is ~25 ng/lane under non-reducing and reducing conditions. Chemiluminescent detection substrate will increase sensitivity by 5 to 50 fold.

ELISA: a working concentration of 0.5-1.0 μg/mL is recommended to detect FGF-8b and FGF-8c. The detection limit for rmFGF-8b is ~1 ng/well.

Immunohistochemistry: a minimum working concentration of 25 μg/mL is recommended to detect FGF-8 in paraffin-embedded human prostate cancer tissue sections.

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 <0.1 EU (endotoxin units) per 1 μg as determined by the LAL (Limulus amebocyte lysate) method.

References

1. Gemel, J., et al., *Genomics*, **35**, 253 (1996).
2. Givol, D., and Yayon, A., *FASEB. J.*, **6**, 3362 (1992).
3. Baird, A., et al., *Curr. Opin. Neurobiol.*, **4**, 78 (1994).
4. Fernig, F.G., and Gallagher, J.T., *Prog. Growth Factor Res.*, **5**, 353 (1994).
5. Kirkoshi, J., et al., *Biochem. Biophys. Res. Commun.*, **274**, 337 (2000).
6. Nishimura, T., et al., *Biochem. Biophys. Acta.*, **1492** 203 (2000).
7. Bernard, O., and Matthew, P., Guidebook to Cytokines and Their Receptors, (Oxford Press, New York, 1994).
8. Galzie, Z., et al., *Biochem. Cell Biol.*, **75**, 669 (1997).
9. Callard, R., and Gearing, A., The Cytokine Facts Book, (Academic Press, New York, 1994).
10. Tanaka, A., et al., *Proc. Natl. Acad. Sci. USA*, **89**, 8928 (1992).
11. Crossley, P.H., and Martin, G.R., *Development*, **121**, 439 (1995).
12. MacArthur, et al., *Development*, **121**, 3603 (1995).
13. Valve, E., et al., *Biochem. Biophys. Res. Commun.*, **232**,173 (1997).
14. Rizzino, A., et al., *Cancer Res.*, **48**, 4266 (1988).
15. Thomas, K., et al., *Methods Enzymol.*, **147**, 120 1987.

KAA,PHC 08/06-1