Invitrogen

Basic Fibroblast Growth Factor (bFGF), Human, Recombinant

Cat. No.: 13256-029

Size: 10 µg Store at -20°C.

Form: Lyophilized

Storage:

Six months at -20°C as received. Up to six months at -20°C when aliquoted into solution containing carrier protein (see Directions for Use). NOTE: DO NOT STORE IN DILUTE AQUEOUS SOLUTION. DO NOT FREEZE/THAW REPEATEDLY.

Description:

This recombinant preparation of human basic FGF (bFGF) is supplied as a lyophilized powder. It is suitable for use in receptor binding, transmembrane signaling and other cell biology research applications.

Background:

Basic FGF (heparin binding growth factor-2, basic brain-derived growth factor) is a 17-kDa member of the family of heparin binding growth factors, which also includes acidic FGF (HBGF-1) as well as the oncoproteins Int-2 (HBGF-3), HST/K53 (HBGF-4) and HBGF-5 (1). All are potent inducers of DNA synthesis in a variety of normal diploid mammalian cell types from mesoderm and neuroectoderm as well as in established cell lines (2). Heparin has been shown to potentiate the biological activity of acidic FGF (3), but does not augment the mitogenic activity of bFGF (4,5). The serum protein α_2 -macroglobulin has recently been shown to bind bFGF and may inactivate this and other growth factors *in vivo* (6).

Doc. Rev. 121701

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Basic FGF has been found in or associated with a variety of solid tissues, tumors and cultured cells (4,7). *In vivo*, bFGF has been shown to be a potent angiogenic agent (9,16). Recent *in vitro* studies show that bFGF can bind to heparin-like molecules in the extracellular matrix (ECM) of endothelial cells (10). The interaction of bFGF with components of the ECM such as type IV collagen and fibronectin, may serve to locally regulate endothelial cell growth and differentiation during angiogenesis (11).

Basic FGF and acidic FGF are structurally similar (7) but functionally different (8). There are four cysteine residues in bFGF, two of which are conserved among all members of the HBGF family. The two remaining cysteines are not essential for biological activity (7). Basic FGF has two sequences that are characteristic of heparin-binding domains. However, synthetic peptides including flanking sequences also bind to heparin, suggesting that the heparin-binding activity of bFGF is not restricted to simple domains (12).

Basic and acidic FGF are mitogenic for the same cell types, suggesting that they interact with the same receptors (13). Comparison of neuronal and mesenchymal receptors for bFGF reveals size and structure similarities, but functional differences (14). Radioreceptor assays for bFGF demonstrate the existence of saturable, high affinity binding sites on a variety of cell types ($K_d = 10-200 \text{ pM}$; 0.2-10 x 10⁴ binding sites/cell). Cells also have low affinity binding sites which appear to be cell-associated heparin-like molecules (1).

Cell growth regulation by FGFs is complex. Besides its well described mitogenic effects on fibroblasts (10, 11), bFGF has been shown to inhibit EGF receptor binding in murine 3T3 cells (15). Further evidence for complex regulation of FGF activity is suggested by the finding that down regulation of FGF receptors correlates with the transformed phenotype(16).

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APPLICATIONS:

- 1. Studies of angiogenesis (8,11)
- 2. Studies of mitogenesis of fibroblasts (10,11)
- 3. Neurite outgrowth studies in PC12 cells (17)
- 4. Receptor binding studies (13-16)
- 5. Tyrosine phosphorylation studies (18)
- 6. Protein kinase phosphorylation (PKC, PKA) (19,20)

DIRECTIONS FOR USE: Reconstitute in 100 µl of 10 mM Tris, pH 7.6, to yield a stock solution of 0.1 mg/ml of bFGF. To avoid loss due to adsorption, prepare dilute solutions in appropriate assay buffer containing at least 0.1% BSA just prior to use. Do not store in dilute solution. For longer term storage, aliquot into buffer containing 0.1% BSA and store in polypropylene vials at -20°C. Avoid repeated freezing and thawing. In applications requiring long term use of this growth factor in cell cultures, it is advisable to refilter material after dilution in BSA-containing buffer, through a 0.22 micron low protein-binding filter.

QUALITY CONTROL:

PURITY

- SDS-PAGE: ≥95% purity
- ENDOTOXIN: $\leq 0.1 \text{ ng/}\mu\text{g}$

FUNCTIONAL QUALIFICATION

THYMIDINE INCORPORATION: ED_{50} ≤ 0.5 ng/ml as determined by the dose dependent stimulation of thymidine uptake by BaF3 cells expressing FGF receptors.

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