Recombinant Human FGF basic/FGF2/bFGF GMP

Catalog Number: 3718-GMP

DESCRIPTION

Source	*E. coli*-derived human FGF basic/FGF2/bFGF protein Pro143-Ser288 Accession # NP_001997.5 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	Pro_{143}-Ala-Leu-Pro-Glu-Asp-Gly-Gly-Ser-Gly Ala_{144}-Leu-Pro-Glu-Asp-Gly-Gly-Ser-Gly-Ala
Predicted Molecular Mass	16 kDa

SPECIFICATIONS

Activity	Measured in a cell proliferation assay using NR6R-3T3 mouse fibroblast cells. Raines, E.W. *et al.* (1985) Methods Enzymol. **109**:749. The ED_{50} for this effect is 0.1-0.6 ng/mL. The specific activity of Recombinant Human FGF basic/FGF2 GMP is >8.0 x 10^5 IU/mg, which is calibrated against the human FGF basic/FGF2 WHO International Standard (NIBSC code: 90/712).
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE with silver staining, under reducing conditions.
Host Cell Protein	< 0.5 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative for Mycoplasma.
Formulation	Lyophilized from a 0.2 µm filtered solution in Tris-HCl and NaCl. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100-200 µg/mL in PBS.
Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 3 months, ≤ -20 °C under sterile conditions after reconstitution.

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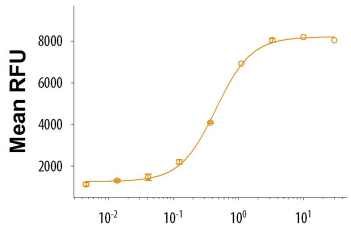
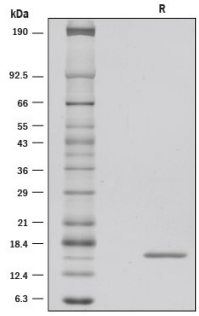
DATA

Bioactivity



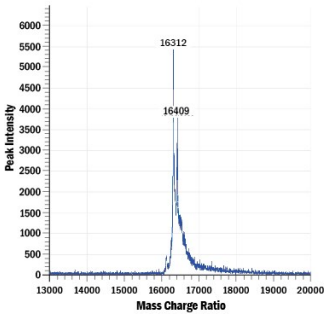
Recombinant Human FGF basic/FGF2 (145 aa) GMP Protein Bioactivity GMP-grade Recombinant Human FGF basic/FGF2 (Catalog # 3718-GMP) stimulates proliferation of the NR6R-3T3 mouse fibroblast cell line. The ED_{50} for this effect is 0.1-0.6 ng/mL.

SDS-PAGE



Recombinant Human FGF basic/FGF2 (145 aa) GMP Protein SDS-PAGE 1 µg/lane of Recombinant Human GMP-grade FGF basic/FGF2 was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a single band at 16 kDa.

Mass Spectrometry



Recombinant Human FGF basic/FGF2 (145 aa) GMP Protein Mass Spectrometry MALDI-TOF analysis of GMP-grade Recombinant Human FGF-basic/FGF2 (Catalog # 3718-GMP). The peaks at 16312 and 16409 correspond to the measured molecular weight of the intact protein. The calculated mass is 16311 Da for Ala144-Ser288 and 16408 Da for Pro143-Ser288.

BACKGROUND

FGF basic (also known as FGF2 and HBGF-2) is an 18-34 kDa, heparin-binding member of the FGF superfamily of molecules (1-3). Superfamily members are characterized by the presence of a centrally placed β-trefoil structure. FGF acidic (FGF-1) and FGF basic (FGF2) were the first two identified FGFs, and the designations acidic and basic refer to their relative isoelectric points. Human FGF basic is 288 amino acids (aa) in length. There are multiple start sites, four of which utilize atypical CUG codons, and one that initiates at an AUG start site (4 - 6). The four CUG start sites generate high molecular weight (HMW) FGF basic. There is a 34 kDa, 288 aa form, a 24 kDa, 210 aa form, a 22.5 kDa, 201 aa form, and a 22 kDa, 196 aa form. All are retained intracellularly, undergo extensive methylation, and possess one or more nuclear localization signals (NLS) (7-9). The AUG initiating form is 18 kDa and 155 aa in length. There is no signal sequence (ss). It is, however, secreted directly through the plasma membrane via a mechanism that appears to be dependent upon tertiary structure (10). In place of a ss, there is purportedly a 9 aa N-terminal prosegment that precedes a 146 aa mature segment (11). Early isolations of 18 kDa bovine FGF basic yielded 146 aa molecules, an effect attributed to the presence of acid proteases (12). The molecule contains a heparin-binding site (aa residues 128-144), and undergoes phosphorylation at Ser117 (13). There is also an ill-defined C-terminal NLS that may be more “functional” (or 3-dimensional) than structural (7). Human 146 aa FGF basic is 97% aa identical to mouse FGF basic (14).

References:

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