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### 2. 1. Product Information

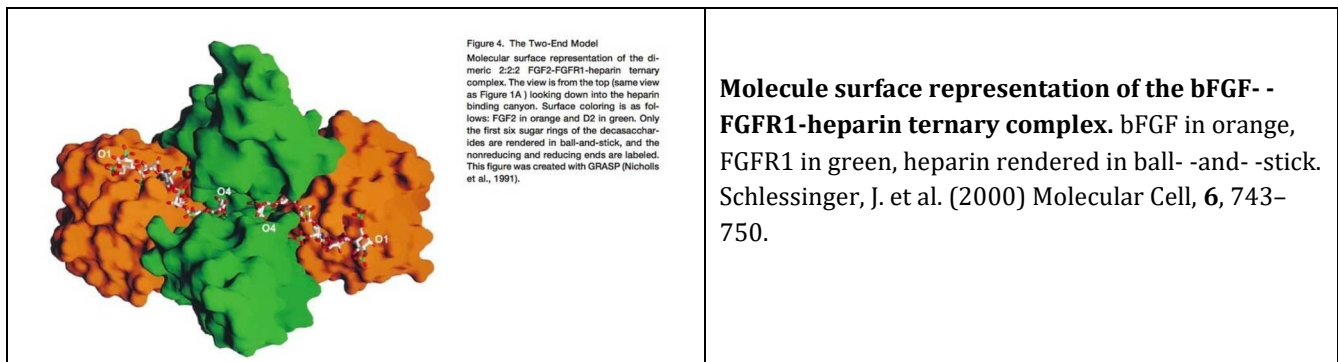
#### 1.1. Background

Recombinant human FGF-basic, also known as FGF-2 or bFGF, is a heparin-binding member of the FGF superfamily of at least 23 related mitogenic proteins. bFGF has been isolated from a number of sources, including neural tissue, pituitary, adrenal cortex, corpus luteum, and placenta. It is also involved in a number of biological processes including embryonic development, differentiation, survival, regeneration, migration, wound healing and tissue repair (ref:1-5). bFGF is a critical factor for growing embryonic stem cells in culture to remain in an undifferentiated state.

A variety of forms of FGF basic are produced as different N-terminal extensions. These extensions of N-terminal affect localization of FGF basic in cellular compartments but do not affect biological activity. bFGF contains four cysteine residues. Two of them exist in free sulfhydryl form. bFGF is an instable molecule during storage and delivery.

#### 1.2 bFGF, Heparin and FGFR

The biological activities of bFGF are presumably mediated through specific, high affinity cell surface receptors present on a variety of cells. Binding of FGF to heparin or cell surface heparin sulfate proteoglycans is necessary for binding of FGF to high affinity FGF receptors. Additional studies have shown that heparin acts to increase the affinity and half-life of the FGF/FGFR complex.



## 1.3 Applications

bFGF can be used for a variety of applications, including:

- Stimulation of proliferation and differentiation of several cell types, such as mesenchymal stromal cells, neural cells, and endothelial cells.
- Long-term maintenance and propagation of undifferentiated embryonic and induced pluripotent stem cells in reprogramming experiments.
- Differentiation of neural cells starting from embryonic and induced pluripotent stem cell cultures.

For most in vitro applications, bFGF exerts its biological activity in the concentration range of 0.1 to 10.0 ng/mL. Human ES cells require concentrations in the range of 4 to 100 ng/mL, depending on the method of culture. Optimal working concentration is must determined by user.

## 1.4 Specifications

Size	10 µg, 25 µg, 50 µg, 100 µg, 250 µg, 1.0 mg
Express source	Escherichia Coli.
Species	Human
Amino Acid Sequence	AAGSITTLP ALPEDGGSGA FPPGHFKDPK RLYCKNGGFF LRIHPDGRVD GVREKSDPHI KLQLQAEERG VVSIKGVCAN RYLAMKEDGR LLASKCVTDE CFFFERLESN NYNTYRSRKY TSWYVALKRT GQYKLGSKTG PGQKAILFLP MSAKS
Molecule Mass	16.4 kDa
Primary Structure	Single, non-glycosylated polypeptide chain
Purification	Sequential chromatography ED <sub>50</sub> = 0.1-0.3 ng/ml as determined by the dose dependent proliferation of NIH 3T3 cells (figure-2,3).
Purity	>98% as determined by HPLC and SDS page prior to the addition of BSA.
Endotoxin Level	< 0.10 EU per 1 µg of bFGF (0.01 ng/µg) as determined by the LAL assay.
Bioactivity	ED <sub>50</sub> = 0.1-0.3 ng/ml as determined by the dose dependent proliferation of NIH 3T3 cells (figure-2,3).

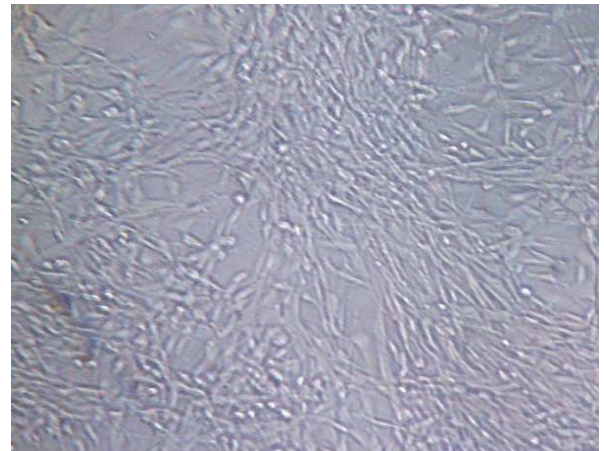
**1.5 Experiment data**

Cell Culture of NIH/3T3 Cells



**FIGURE 2a** NIH 3T3 cells (Mouse embryonic fibroblast cell line, ATCC: CRL-1658) were cultured before adding bFGF.

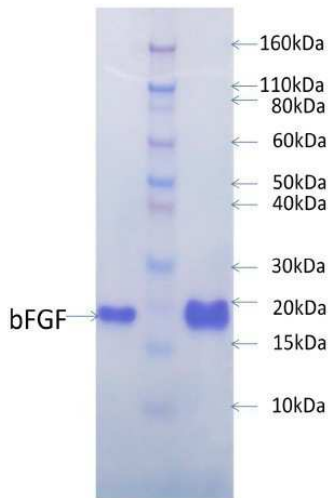
NIH/3T3 cells (mouse fibroblast cell line, ATCC: CCL-1668) were cultured in the absence of bFGF.



**FIGURE 2b** NIH 3T3 cells were cultured with 0.25 ng/ml in serum-free medium at 37 °C, 5% CO<sub>2</sub> for 24 hours.

NIH/3T3 cells were cultured with 0.25 ng/ml bFGF in serum free medium at 37°C, 5% CO<sub>2</sub> for 24 hours.

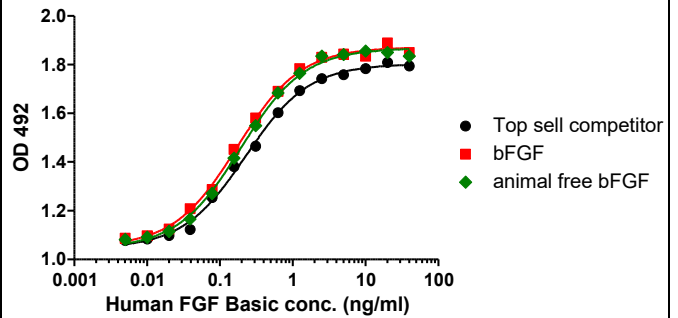
**SDS-PAGE of Human bFGF**



**FIGURE 1**

SDS-PAGE gel of Recombinant Human bFGF Sample

**Bioactivity Assay**



Human FGF basic recombinant protein bioactivity measured by mouse BALB/c 3T3 cell proliferation assay. The cell proliferation dose curves were plotted with mean OD vs. bFGF concentrations (ng/mL).

## 1.6 Different Formulations of bFGF

### 1.6-1: Ready-To-Use bFGF

Catalog Number: 12-0005CFR	Catalog Number: 12-0005CR	Catalog Number: 12-0005HR
<p><b>Formulation I-carrier free:</b></p> <p>Sterile filtered in Tris-HCl and NaCl.</p>	<p><b>Formulation II-with BSA:</b></p> <p>Sterile filtered in Tris-HCl and NaCl with BSA as a carrier protein.</p>	<p><b>Formulation III-with Heparin:</b></p> <p>Sterile filtered in Tris-HCl, NaCl with Heparin as a partner.</p>
<p><b>Shipping:</b></p> <p>The product is shipped on dry ice.</p>	<p><b>Shipping:</b></p> <p>The product is shipped on dry ice.</p>	<p><b>Shipping:</b></p> <p>The product is shipped on dry ice.</p>
<p><b>Reconstitution:</b></p> <p>Centrifuge vial before opening. Reconstitute at 100 µg/ml in sterile PBS.</p>	<p><b>Reconstitution:</b></p> <p>Centrifuge vial before opening. Reconstitute at 100 µg/ml in sterile PBS containing at least 0.5% BSA.</p>	<p><b>Reconstitution:</b></p> <p>Centrifuge vial before opening. Reconstitute at 100 µg/ml in sterile PBS.</p>
<p><b>Storage and stability:</b></p> <p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> <li>• 18 months from date of receipt at -20 to -80 °C.</li> <li>• 12 months at -20 to -80 °C under sterile conditions after reconstitution.</li> <li>• Two weeks at 2 to 8 °C under sterile conditions after reconstitution.</li> </ul>	<p><b>Storage and stability:</b></p> <p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> <li>• 18 months from date of receipt at -20 to -80 °C.</li> <li>• 12 months at -20 to -80 °C under sterile conditions after reconstitution.</li> <li>• 1 month at 2 to 8 °C under sterile conditions after reconstitution.</li> </ul>	<p><b>Storage and stability:</b></p> <p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> <li>• 18 months from date of receipt at -20 to -80 °C.</li> <li>• 12 months at -20 to -80 °C under sterile conditions after reconstitution.</li> <li>• 1 month at 2 to 8 °C under sterile conditions after reconstitution.</li> </ul>

1.6-2: Lyophilized bFGF

Catalog Number: 12-0005CFL	Catalog Number: 12-0005CL	Catalog Number: 12-0005HL
<p><b>Formulation I-carrier free:</b></p> <p>Sterile filtered and lyophilized in Tris-HCl and NaCl.</p>	<p><b>Formulation II-with BSA:</b></p> <p>Sterile filtered and lyophilized in Tris-HCl and NaCl with BSA as a carrier protein.</p>	<p><b>Formulation III-with Heparin:</b></p> <p>Sterile filtered and lyophilized in Tris-HCl, NaCl with Heparin as a partner.</p>
<p><b>Shipping:</b></p> <p>The product is shipped at ambient temperature with ice bags. Upon receipt, it should be stored immediately at -20 to -80 °C.</p>	<p><b>Shipping:</b></p> <p>The product is shipped at ambient temperature with ice bags. Upon receipt, it should be stored immediately at -20 to -80 °C.</p>	<p><b>Shipping:</b></p> <p>The product is shipped at ambient temperature with ice bags. Upon receipt, it should be stored immediately at -20 to -80 °C.</p>
<p><b>Reconstitution:</b></p> <p>Centrifuge vial before opening. Reconstitute at 100 µg/ml in sterile PBS.</p>	<p><b>Reconstitution:</b></p> <p>Centrifuge vial before opening. Reconstitute at 100 µg/ml in sterile PBS containing at least 0.5% BSA.</p>	<p><b>Reconstitution:</b></p> <p>Centrifuge vial before opening. Reconstitute at 100 µg/ml in sterile PBS.</p>
<p><b>Storage and stability:</b></p> <p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> <li>• 18 months from date of receipt at -20 to -80 °C.</li> <li>• 12 months at -20 to -80 °C under sterile conditions after reconstitution.</li> <li>• Two weeks at 2 to 8 °C under sterile conditions after reconstitution.</li> </ul>	<p><b>Storage and stability:</b></p> <p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> <li>• 18 months from date of receipt at -20 to -80 °C.</li> <li>• 12 months at -20 to -80 °C under sterile conditions after reconstitution.</li> <li>• 1 month at 2 to 8 °C under sterile conditions after reconstitution.</li> </ul>	<p><b>Storage and stability:</b></p> <p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> <li>• 18 months from date of receipt at -20 to -80 °C.</li> <li>• 12 months at -20 to -80 °C under sterile conditions after reconstitution.</li> <li>• 1 month at 2 to 8 °C under sterile conditions after reconstitution.</li> </ul>

## 1.6-3: Animal Free bFGF

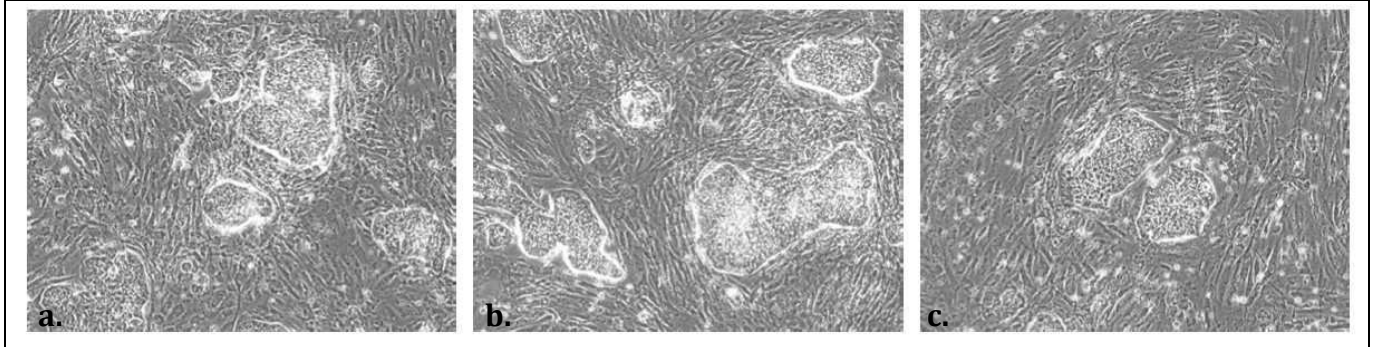
Catalog Number: 12-0005AFR	Catalog Number: 12-0005AFL
<b>Formulation I-ready to use:</b> Sterile filtered in Tris-HCl and NaCl.	<b>Formulation I-lyophilized:</b> Sterile filtered and lyophilized in Tris-HCl and NaCl.
<b>Shipping:</b> The product is shipped on dry ice.	<b>Shipping and Reconstitution:</b> The product is shipped at ambient temperature with ice bag. Upon receipt, it should be stored immediately at -20 to -80 °C. Centrifuge vial before opening. Reconstitute at 100 µg/ml in sterile PBS.
<b>Storage and stability:</b> Use a manual defrost freezer and avoid repeated freeze-thaw cycles.  <ul style="list-style-type: none"> <li>• 12 months from date of receipt at -20 to -80 °C.</li> </ul> Two weeks at 2 to 8 °C under sterile conditions.	<b>Storage and stability:</b> Use a manual defrost freezer and avoid repeated freeze-thaw cycles.  <ul style="list-style-type: none"> <li>• 18 months from date of receipt at -20 to -80 °C.</li> <li>• 12 months at -20 to -80 °C under sterile conditions after reconstitution.</li> <li>• Two weeks at 2 to 8 °C under sterile conditions after reconstitution.</li> </ul>

## 1.6-4 Select right formulation for your experiment

Human bFGF is not a stable growth factor as it contains free cysteine residues and has several protein interaction surfaces on its 3D structure. It is more stable in the presence of BSA as a carrier. We suggest purchasing the protein with BSA for use in cell, tissue culture and stem cell reprogramming. The bFGF in complex with heparin increases the stability of bFGF and enhances its affinity with FGF receptor (6). We advise using the formulation for cell culture that needs strong bFGF affinity in the presence of low concentration of heparin. In addition, heparin should be a positive factor for cell culture of epithelial cells, neural stem and progenitor cell. The carrier free protein is recommended for application if BSA and heparin interfere with your experiment.



bFGF of three formulations were used in stem cell reprogramming project that generated pluripotent colonies from fibroblasts. They show similarly good results to produce iPS cells.



a. Using carrier free formulation b. Using BSA formulation c. Using heparin formulation.

## 1.7 Features of Recombinant Protein From Aurora Biolabs

**High purity**- frees interference from other biomolecules, clear data will be obtained.

**High biological activity**-less protein, better data from your assay.

**Low endotoxin**- endotoxins strongly interfere with cell function and growth, we provide cytokines with endotoxin level less than 0.1 EU/ $\mu$ g.

**High stability and consistency**- reliable data in an inexpensive and a time efficient manner.

## 2. References:

1. Dvorak, P. and Hampl, A. (2005) *Folia Histochem Cytobiol* 43, 203-8.
2. Ornitz, D.M. and Itoh, N. (2001) *Genome Biol* 2, REVIEWS3005.
3. Shi, Y. et al. (2008) *Crit Rev Oncol Hematol* 65, 43-53.
4. Fontijn, D. et al. (2006) *Br J Cancer* 94, 1627-36.
5. Marek, L. et al. (2009) *Mol Pharmacol* 75, 196-207.
6. Ornitz, D.M. et al. (1992) *Mol Cell Biol*.12, 240-7.



bFGF sample will be shipped  
in our cryopro storage box.