Performance Report on Future Fields' Recombinant Bovine FGF2

Empowering Life Science Innovators

At Future Fields, our utmost passion lies in assisting you in reaching your research objectives. We understand that you are confronted with ever-evolving challenges, and the burden of costly and unreliable recombinant proteins should not be one of them. Hence, our products have been meticulously crafted with a focus on quality, affordability, and sustainability—ensuring that these advantages are transferred directly to you, the dedicated scientist.

We are excited to provide you an in depth look at the performance characteristics of our recombinant protein product: **Bovine FGF2 (bvFGF2)**. As an animal species growth factor (Figure 1) with in vitro and in vivo applications, we ensure that the identity, functionality and quality of the proteins that are reaching you are of the highest standards. Below, we lay out the numerous cell-based and biophysical technique based assays that were carried out to ascertain the functionality and quality of bovine FGF2 produced using the EntoEngine™ technology.

Figure 1 - Ribbon Structure of Bovine FGF2

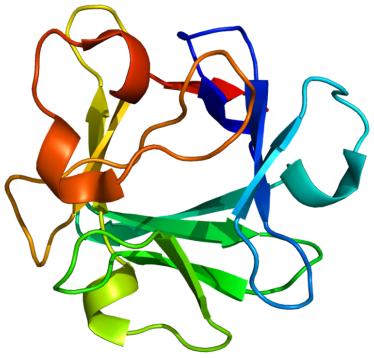


Figure 1: Ribbon structure of bvFGF2 showing β barrel tertiary structure consisting of 12 antiparallel β strands connected by β turns.

As with many growth factors, it is important to ascertain their appropriate folding and conformation to ensure the proteins are able to bind to their cognate receptors, trigger a cell signaling pathway and ultimately lead to an expected outcome in cell behavior (for example, proliferation).

We go above and beyond in our production of recombinant protein by using the latest purification techniques and lot release testing. These are paired with our Quality Management System to ensure the effectiveness and consistency of our affordable growth factors.
In addition to making quality and functional proteins, we know your concerns for scalability are paramount. With the inherent advantages of our EntoEngine™ platform, we are able to meet your bigger demands in short turnaround time to support cutting edge research in cellular agriculture, in vitro diagnostics, in vivo animal studies and even ex vivo reproduction biology applications.

Here is a brief summary of the report:

1. **Performance:** Functionality of bvFGF2 validated using multiple metrics
   - 1.1. SPR readout confirming protein identity and binding
     - 1.1.1. Ligand-Antibody interaction (sub-nanomolar binding affinity)
   - 1.2. Cell based assay confirming the functionality of bvFGF2 in cell cultures
     - 1.2.1. Consistent EC_{50} ~ 1-3 ng/ml in cell-based proliferation assay
     - 1.2.2. Stimulation of AKT and MAPK/ERK pathways in NIH-3T3 cells upon stimulation with bvFGF2
     - 1.2.3. Images of NIH-3T3 cells to demonstrate healthy cell morphology when grown with bvFGF2
2. **Purity:** >95 % purity
3. **Safety:**
   - 3.1. Negative for Mycoplasma contamination
   - 3.2. Below safety threshold (< 1 EU/µg) for Endotoxin
4. **Sustainability:**
   - 4.1. ACT Environmental Impact Scores of 24.8 and 28.3
   - 4.2. Other sustainability initiatives

1. Performance

1.1 SPR determined identity and binding affinity of bovine FGF2

Surface plasmon resonance (SPR) is a powerful biophysical technique that can accurately determine the conformity of a target by determining its binding affinity to a given ligand. Here, we use SPR to determine the identity and appropriate folding of bvFGF2 by measuring the ability of the protein to bind to a FGF2 antibody that is immobilized on a surface.

Based on our results, we determined that bvFGF2 has nanomolar affinity (KD 1–2 nM) to the FGF2 antibody, consistently among different vial sizes, thereby confirming the binding affinity and identity of the protein.

1.1.1 Ligand-Antibody interaction (sub-nanomolar binding affinity)

Figure 2 - Future Fields Bovine FGF2 vs. Competitor Human FGF2 Binding to Anti-FGF2 Antibody

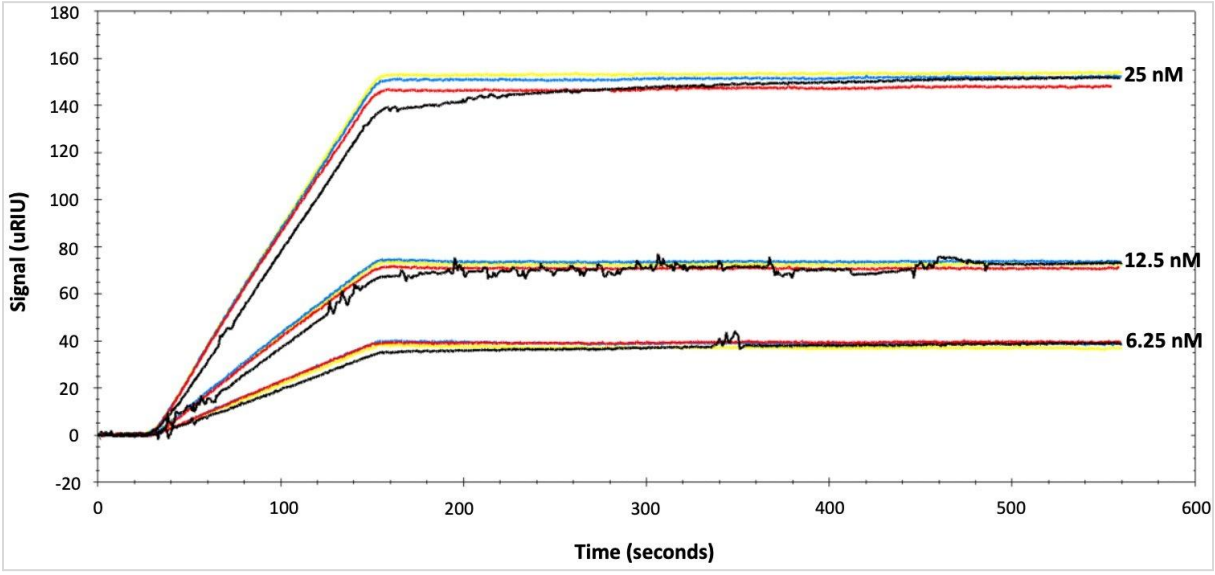


Figure 2: SPR sensorgrams of bvFGF2 binding to immobilized FGF2 antibody on the substrate. Black: competitor FGF2; Red: FF bvFGF2 (50-µg vial); Blue: FF bvFGF2 (100-µg vial); Yellow: FF bvFGF2 (250-µg vial). KD was determined to be between 1–2 nM for both competitor's and Future Fields bvFGF2.

1.2 Cell based assay confirming the functionality of bvFGF2 in cell cultures

Did you know?

NIH-3T3 mouse embryonic fibroblast cells are so named to indicate a cell culture methodology: 3-day transfer, inoculum 3 x 105 cells.

We used a number of methods to assess the functionality and biological properties of bvFGF2. One of them is using cell based proliferation assays to determine the ability of FGF2 to drive population growth. We used alamarBlue[1] colorimetric readout. This assay follows the cellular metabolic activity as a measure of viable proliferating cells (Figure 3); put simply, healthy and actively dividing cells are expected to have higher metabolic rate than cells that are either stressed, undergoing apoptosis or stagnant. This increase in metabolic rate can be measured using a chemical reagent resazurin (Blue), which turns into resorufin (Red) when metabolized in the mitochondria.

[1] alamarBlue™ HS Cell Viability Reagent (thermofisher.com)

1.2.1 Bovine FGF2 drives NIH-3T3 cell proliferation

Figure 3 - Assay Diagram Demonstrating Metabolic Activity

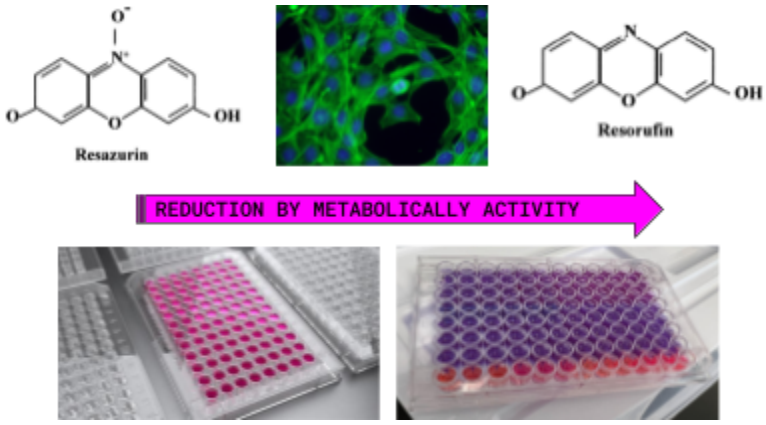


Figure 3: Representing the chemical conversion of non-fluorescent blue resazurin to pink fluorescent resorufin in the cells. This colorimetric assay can be performed in a high-throughput setup.

We tested Future Fields' recombinant bvFGF2 and competitor FGF2 for their ability to stimulate the proliferation of NIH-3T3 mouse fibroblast cells in a dose-dependent manner (**EC_{50} = 1-3 ng/ml**). We observed highly comparable results for different lots of Future Fields' bvFGF2; to provide assurance of our product's performance, every lot we generate goes through a vigorous set of manufacturing lot-release assays and only lots that are compliant to our high standards are sent to our clients.

Figure 4 - % of Proliferation of NIH-3T3 Cells Stimulated with Bovine FGF2

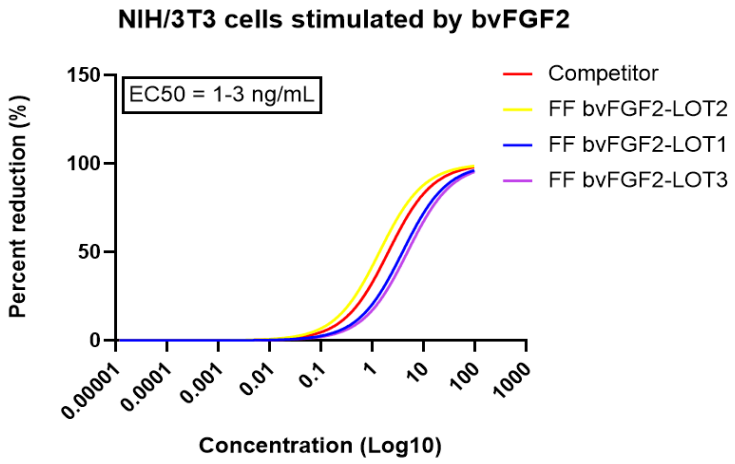


Figure 4 - Graph represents multiple lots of Future Fields' bvFGF2 tested for their ability to drive proliferation of NIH-3T3 cells, using alamarBlue assay, in comparison to competitor standard. EC_{50} range: 1-3 ng/mL

1.2.2 Bovine FGF2 activates proliferation signals in cells via MAPK/ERK pathway

The growth factor bvFGF2 signals via transmembrane Receptor Tyrosine Kinases-FGF-receptor; upon binding of ligand to its receptor, MAPK/ERK and AKT pathways are triggered in the cell that results in proliferation signals, gene expression and cycle progression. Phosphorylation of ERK1/2 and its nuclear translocation is a key event in this pathway, and therefore can be used as an indirect measure of FGF2-FGF receptor binding and activation.

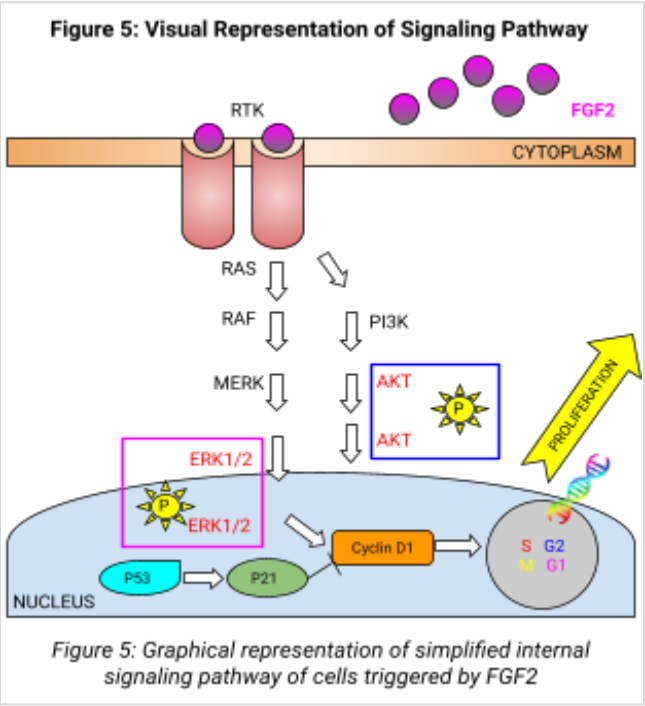


Figure 5: Graphical representation of simplified internal signaling pathway of cells triggered by FGF2

Here, we stimulated serum-starved NIH-3T3 for 10 minutes with different lots of bvFGF2 or competitor FGF2 to ascertain their ability to trigger the ERK and AKT phosphorylations. 10% FBS served as positive control and plain media served as negative control; as well, purification buffer served as an internal negative control. Staining for pAkt (S473)[2], ERK/pERK 1/2[3] confirmed consistent activation of internal signals by bvFGF2 arising from different lots (Figure 6).

Figure 6 - Stimulation of proliferation and survival signals in cells by bvFGF2

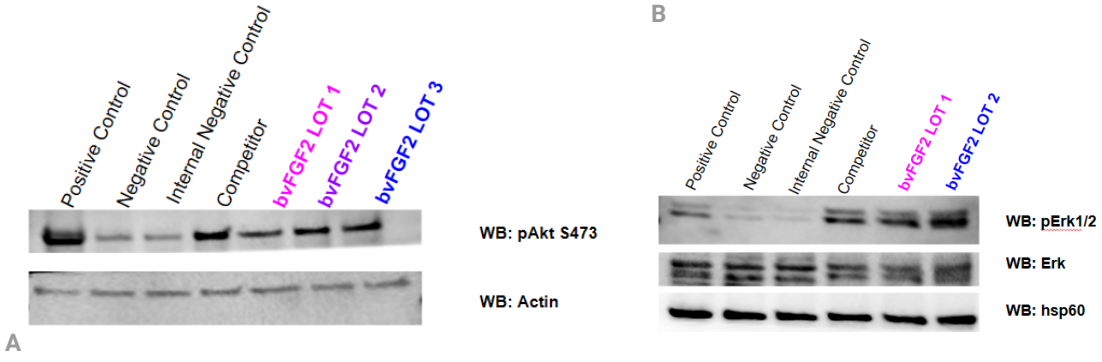


Figure 6 - Serum starved NIH-3T3 cells stimulated with Future Fields' bvFGF2 (different lots), competitor FGF2, 10% FBS or negative controls for 10 minutes, followed by lysis and western blotting techniques to determine A) pAKT (S473) and B) pERK1/2 presence in lysate compared to internal control (actin, total ERK and Hsp60).

[2] Phospho-Akt (Ser473) Antibody | Cell Signaling Technology
[3] p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb | Cell Signaling Technology

1.2.3 Cells cultured with Future Fields Bovine FGF2 shows healthy morphology

Healthy fibroblasts have a distinct morphology: branched cytoplasm surrounding an elliptical, speckled nucleus having two or more nucleoli. Healthy fibroblasts can be recognized by their abundant rough endoplasmic reticulum. Although disjointed and scattered when they have to cover a large space, fibroblasts, when crowded, often locally align in parallel clusters (Figure 7). When stressed by their microenvironment, fibroblasts tend to lengthen their cell body and is a classic marker of unhealthy cells.

When cells are grown with Future Fields bvFGF2, healthy cell morphology is observed for several passages.

Figure 7 - Healthy morphology of NIH-3T3 cells growing with Future Fields bvFGF2

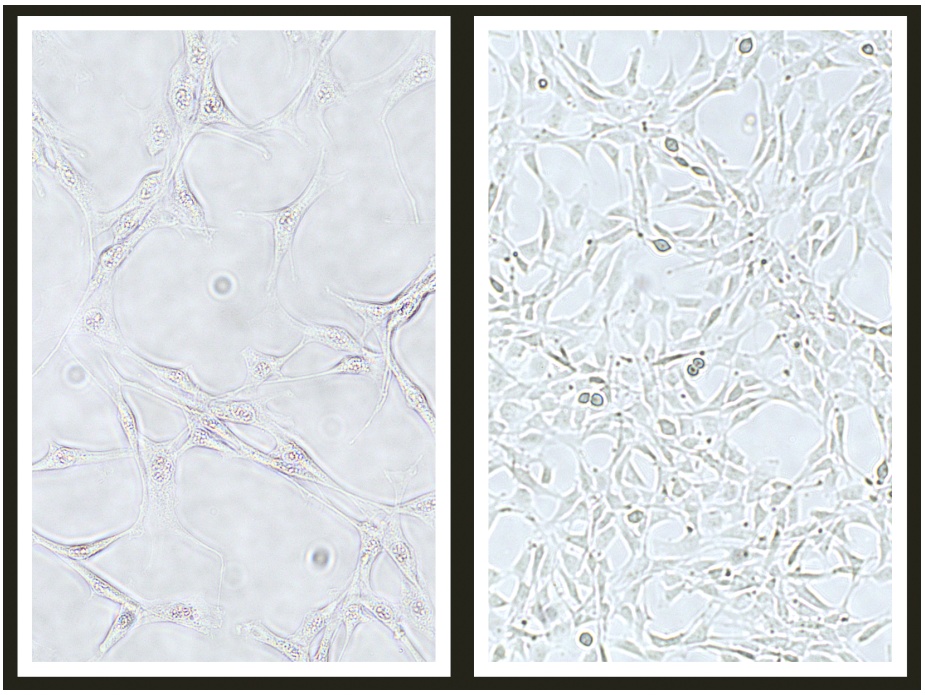


Figure 7: Morphology of NIH-3T3 cells Day 1 (left) and Day 3 (right) post-stimulation with Future Fields' bvFGF2.

2. Purity

In our pursuit of excellence, we deploy a proprietary column-based purification strategy (Figure 8) to ensure the utmost purity of our growth factors. This approach not only preserves the structural integrity and functionality of the product throughout the purification process, but also guarantees minimal to no presence of host components in the final product. As demonstrated in Figure 8, Future Fields' bovine FGF2 consistently achieves a purity level surpassing 95% as visualized by SYPRO stained SDS PAGE gels.

Figure 8 - Purity of bvFGF2 as determined by SDS PAGE

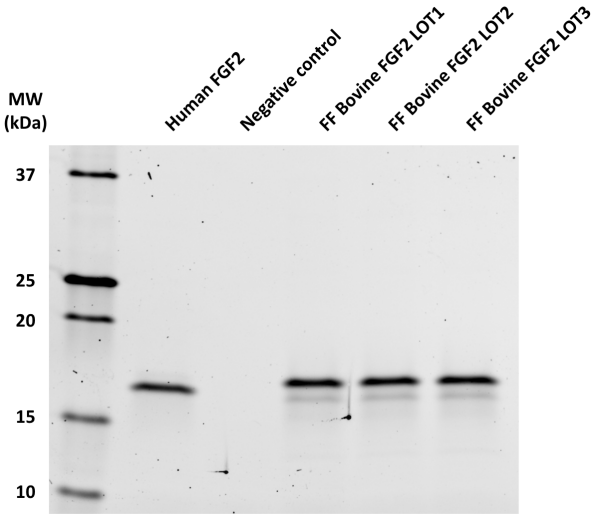


Figure 8 - SDS PAGE gel showing positive control (human FGF2) from competitor and different lots of bvFGF2 for their purity

3. Safety

3.1 Mycoplasma testing

We prioritize the safety of your cell culture and animal-based applications. Understanding the significance of contaminant-free growth factors and media reagents, we take diligent measures to test our products for *Mycoplasma*, a troublesome intracellular antibiotic-resistant contaminant. To provide you with utmost confidence, our proteins undergo comprehensive testing for several species of *Mycoplasma, Acholeplasma, and Ureaplasma* using a highly sensitive PCR-based test (Figure 9). This state-of-the-art testing method can detect as little as 1-5 fg of *Mycoplasma* DNA, corresponding to 2-5 *Mycoplasma* per sample volume, ensuring that the products we deliver are free from these detrimental contaminants.

Figure 9 - PCR-based Mycoplasma contamination check in bvFGF2 lot

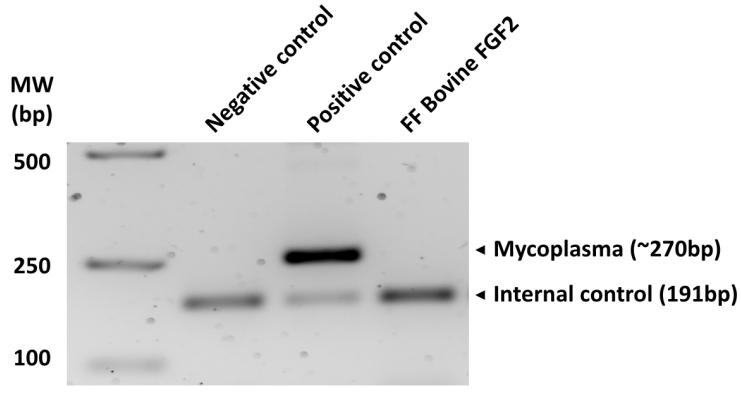


Figure 9 - Mycoplasma contamination was tested by PCR-based assay. DNA gel shows positive control (Mycoplasma DNA), negative control (buffer only) and Future Fields' bvFGF2 confirming the lack of any contaminants.

3.2 Endotoxin testing

To uphold our commitment to quality, we subject our products to stringent endotoxin testing. Endotoxins, commonly known as Lipopolysaccharides (LPS) derived from bacteria, can activate apoptotic pathways in mammalian cell cultures, potentially leading to adverse effects. With Future Fields' bovine FGF2, you can be confident that our products consistently exhibit levels below the threshold of other competitors in the space (<1 EU/µg), eliminating concerns of unwanted deleterious cell signaling and ensuring optimal cell culture conditions (Figure 10).

Figure 10 - Endotoxin levels in different bvFGF2 lots

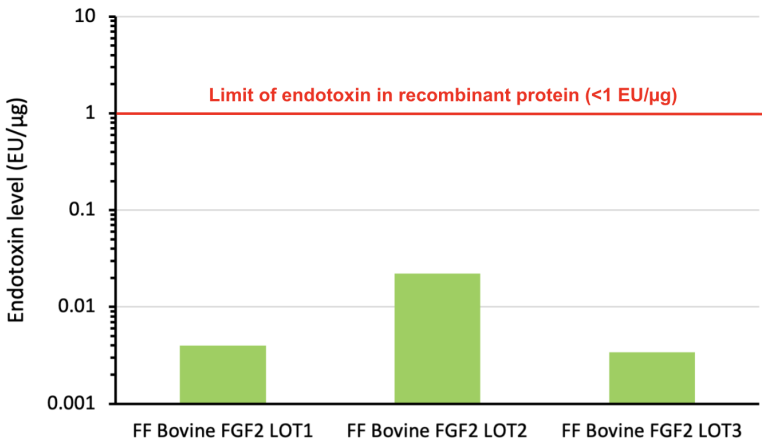


Figure 10 - Represents different lots of bvFGF2 for Endotoxin levels as determined by LAL chromogenic assay; The Endotoxin levels are determined to be much below the expected threshold of 1 EU/µg.

In conclusion, the above characteristics of Future Fields' bovine FGF2 produced by the EntoEngine™ can meet your expectations for consistency of high performance and reliability of supply chain to support your research and production goals.

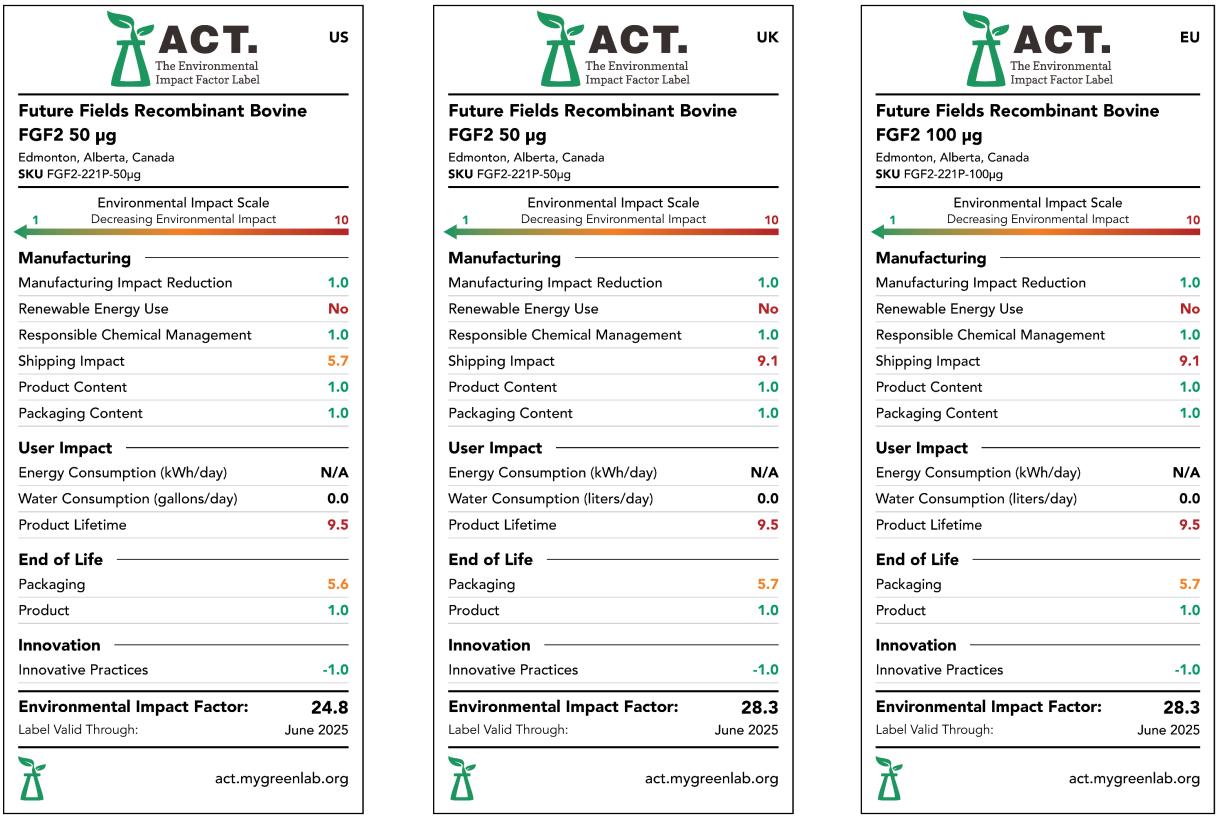
4. Sustainability

4.1 ACT Environmental Impact Factor Label and scores

The ACT Environmental Impact Factor Label from My Green Lab was designed to address the need of both scientists and procurement specialists for clear, third-party verified information about the environmental impact of laboratory products. By emphasizing Accountability, Consistency, and Transparency (ACT) around manufacturing, energy and water use, packaging, and end-of-life, ACT makes it easy for scientists and other biotechnology professionals to choose more sustainable laboratory products.

Future Fields' Recombinant Bovine FGF2 is the first growth factor in the world to receive an ACT Label, scoring 24.8 and 28.3 depending on the shipping destination (Figure 11).

Figure 11 - ACT Labels for Future Fields' Recombinant Bovine FGF2



ACT. The Environmental Impact Factor Label — US

Future Fields Recombinant Bovine FGF2 50 µg
Edmonton, Alberta, Canada
SKU FGF2-221P-50µg

Environmental Impact Scale
Decreasing Environmental Impact (1 – 10)

Manufacturing	
Manufacturing Impact Reduction	1.0
Renewable Energy Use	No
Responsible Chemical Management	1.0
Shipping Impact	5.7
Product Content	1.0
Packaging Content	1.0
User Impact	
Energy Consumption (kWh/day)	N/A
Water Consumption (gallons/day)	0.0
Product Lifetime	9.5
End of Life	
Packaging	5.6
Product	1.0
Innovation	
Innovative Practices	-1.0
Environmental Impact Factor:	**24.8**
Label Valid Through:	June 2025

act.mygreenlab.org

ACT. The Environmental Impact Factor Label — UK

Future Fields Recombinant Bovine FGF2 50 µg
Edmonton, Alberta, Canada
SKU FGF2-221P-50µg

Environmental Impact Scale
Decreasing Environmental Impact (1 – 10)

Manufacturing	
Manufacturing Impact Reduction	1.0
Renewable Energy Use	No
Responsible Chemical Management	1.0
Shipping Impact	9.1
Product Content	1.0
Packaging Content	1.0
User Impact	
Energy Consumption (kWh/day)	N/A
Water Consumption (liters/day)	0.0
Product Lifetime	9.5
End of Life	
Packaging	5.7
Product	1.0
Innovation	
Innovative Practices	-1.0
Environmental Impact Factor:	**28.3**
Label Valid Through:	June 2025

act.mygreenlab.org

ACT. The Environmental Impact Factor Label — EU

Future Fields Recombinant Bovine FGF2 100 µg
Edmonton, Alberta, Canada
SKU FGF2-221P-100µg

Environmental Impact Scale
Decreasing Environmental Impact (1 – 10)

Manufacturing	
Manufacturing Impact Reduction	1.0
Renewable Energy Use	No
Responsible Chemical Management	1.0
Shipping Impact	9.1
Product Content	1.0
Packaging Content	1.0
User Impact	
Energy Consumption (kWh/day)	N/A
Water Consumption (liters/day)	0.0
Product Lifetime	9.5
End of Life	
Packaging	5.7
Product	1.0
Innovation	
Innovative Practices	-1.0
Environmental Impact Factor:	**28.3**
Label Valid Through:	June 2025

act.mygreenlab.org

Figure 11 - ACT labels depicting the Environmental Impact Factor scores for Future Fields' bovine FGF2 for US, UK and EU shipments. Score is 24.8 for US deliveries and 28.3 for UK and EU deliveries. The score does not vary between 50 µg and 100 µg concentrations due to the near-identical processes.

4.2 Other sustainability initiatives

We are a green-certified, bioreactor-free lab. Our EntoEngine™ platform produces recombinant proteins more sustainably and up to 30 times faster than legacy systems in steel tanks.

With Future Fields, you are not just buying a product—you are partnering with a community of forward-thinking researchers who are committed to pushing the limits of what is possible in biotech. We work for humanity and the planet, which is why 1 % of your purchase proceeds will go towards environmental initiatives around the world.

We hope to be a partner in your research success and your champion for positive change.

Get started by requesting a sample today.