Performance Report on Future Fields' Recombinant Human FGF2

Empowering Life Science Innovators

At Future Fields, we are passionate about helping you achieve your research goals. We know that you are constantly facing new challenges, and unreliable recombinant proteins that break the bank should not be one of them. That is why we have designed our products with quality, affordability, and sustainability in mind—to pass on these benefits to you, the scientist.

In this piece, we lay out an in-depth performance analysis of our latest product, Recombinant Human FGF2, and demonstrate its ability to stimulate proliferation in a dose-dependent manner, all the while keeping the cell morphology and other characteristics intact.

Here is a brief summary of the report:

1. **Performance:** Functionality of Human FGF2 validated using multiple metrics
   - 1.1. SPR readout confirming protein identity and binding
     - 1.1.1. Ligand-Receptor interaction (< 5 nM binding affinity) and
     - 1.1.2. Ligand-Antibody interaction (sub-nanomolar binding affinity)
   - 1.2. Consistent EC_{50} ~ 1-3 ng/ml in cell-based proliferation assay
   - 1.3. Fluorescent images to demonstrate healthy cell morphology

2. **Purity:** >95 % purity

3. **Safety:**
   - 3.1. Negative for Mycoplasma contamination
   - 3.2. Below safety threshold (< 1 EU/ug) for Endotoxin

4. **Sustainability:**
   - 4.1. ACT Environmental Impact Scores of 24.8 and 28.3
   - 4.2. Other sustainability initiatives

1. Performance

First, let us start at the heart of our product—its performance. We use multiple methods to qualify our products; biophysical techniques like Surface Plasmon Resonance (SPR) can tell us how tight the binding of the ligand to the substrate (receptor, ligand-specific antibody or other known interactors), as well as cell-based functionality studies to determine that our products work as expected, and they meet your standards for cell culture applications.

1.1 Surface Plasmon Resonance (SPR)

Using FGF-receptor (human), or anti-FGF2 antibody immobilized on a Carboxymethyl Dextran chip, we demonstrate Future Fields' Human FGF2 has an affinity constant of <1 nM on antibody, and ~5 nM on the receptor (Fig. 1). This **nanomolar affinity** indicates a very tight ligand-substrate interaction.

Fig. 1 - Graph of SPR Sensorgram

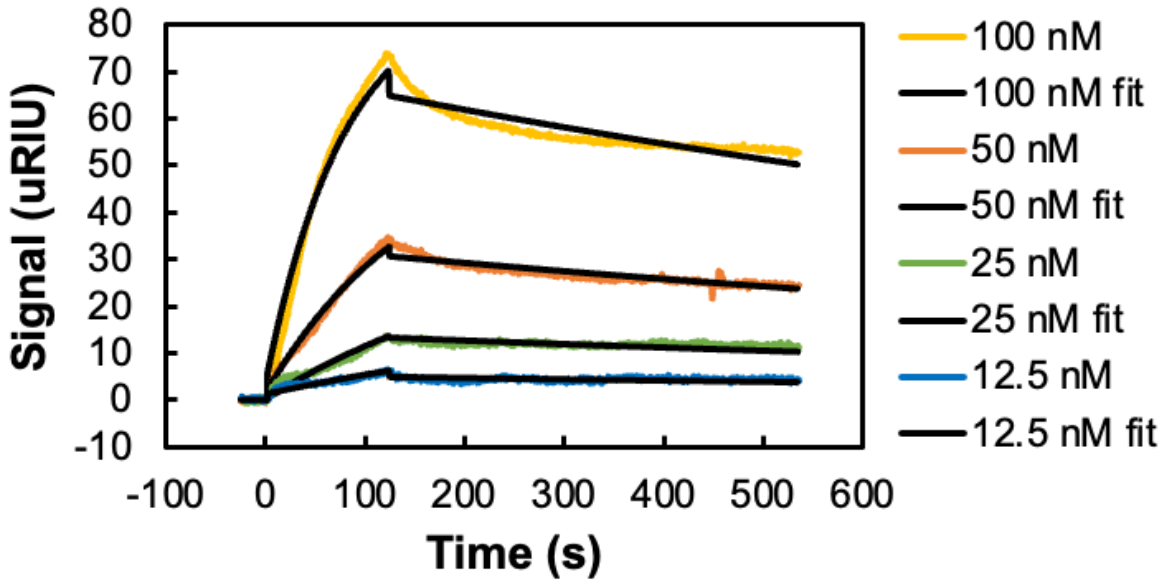

Figure 1: SPR sensorgram showing tight affinity of Human FGF2 on FGF-receptor bound to the substrate. Curve-fit indicates K_d < 5 nM for ligand-receptor interaction.

1.2 Cell-Based Studies (Various Assays)

1.2.1 Proliferation

To characterize Future Fields' Human FGF2 for their functionality, we use multiple cell-based assays. One among them is to assess FGF2 ability to drive proliferation in NIH-3T3 cells (mouse-derived, to demonstrate cross-reactivity). Using a metabolic activity-based readout, we determine that FGF2 is able to drive proliferation in the cells in a dose-dependent manner (**EC_{50} = 1-3 ng/ml**), and is highly comparable to existing competitors' offerings. We also demonstrate lot-to-lot consistency of proteins via cell proliferation, to reduce variability concerns for your research purposes (Fig. 2).

Fig. 2 - % of Proliferation of NIH-3T3 Cells Stimulated with Human FGF2

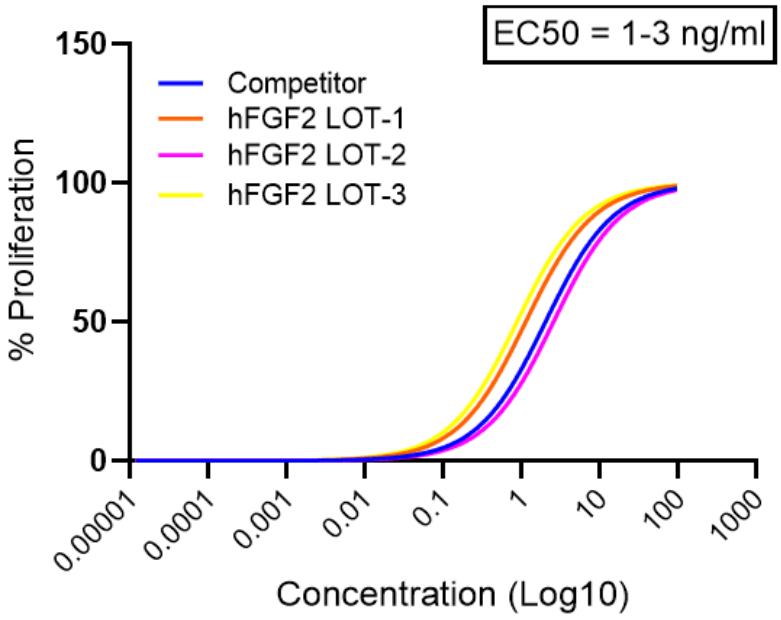

Figure 2: Graph represents multiple lots of Future Fields' Human FGF2 tested for their ability to drive proliferation of NIH-3T3 cells, using alamar blue assay, in comparison to competitor standard. EC_{50} is determined to be in the low nanomolar range indicating active ligands.

1.2.2 Western Blot

As an addition to proliferation studies, we also characterized the downstream signaling pathways triggered in cells when stimulated with Future Fields Human FGF2; for cells to proliferate, critical signaling pathways like the **MAPK/ERK pathway** have to be triggered and activated robustly. Here, we demonstrate lot-to-lot consistency in activating these important proliferation pathways in cells (Fig. 3).

Fig. 3 - Western Blot Probed for pERK to Demonstrate Activation of Proliferation Signals in Cells

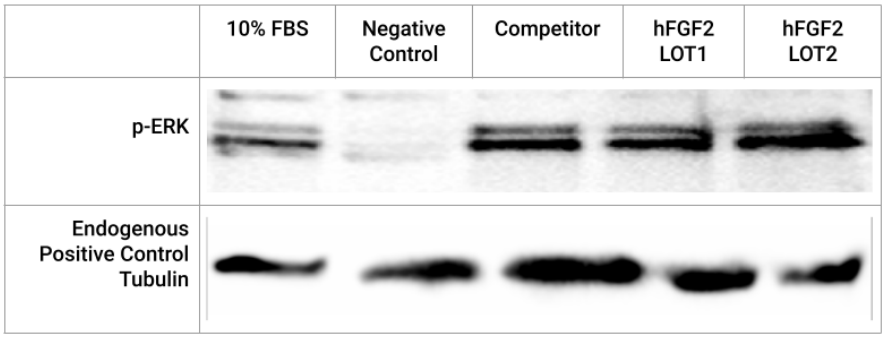

Figure 3: Representative western blots showing stimulation of NIH-3T3 cells with various ligands. Positive controls (FBS and competitor hFGF2) and Future Fields' human FGF2 lots trigger phospho-ERK (p44/42) levels indicating activation of the essential MAPK/ERK pathway in cells. Tubulin acts as an internal loading control.

1.3 Visuals Demonstrating Cell Morphology

1.3.1 Brightfield Cell Images and Population Doubling Time

These representative images (Fig. 4) show cell number increase in time (population doubling time ~20 hours), as well as cell morphology including shape, size, and intact nuclei that affirm that cells at low and high density are **healthy and proliferating robustly.**

Fig. 4 - Graph Showing Cell Proliferation and Images Tracked in Time

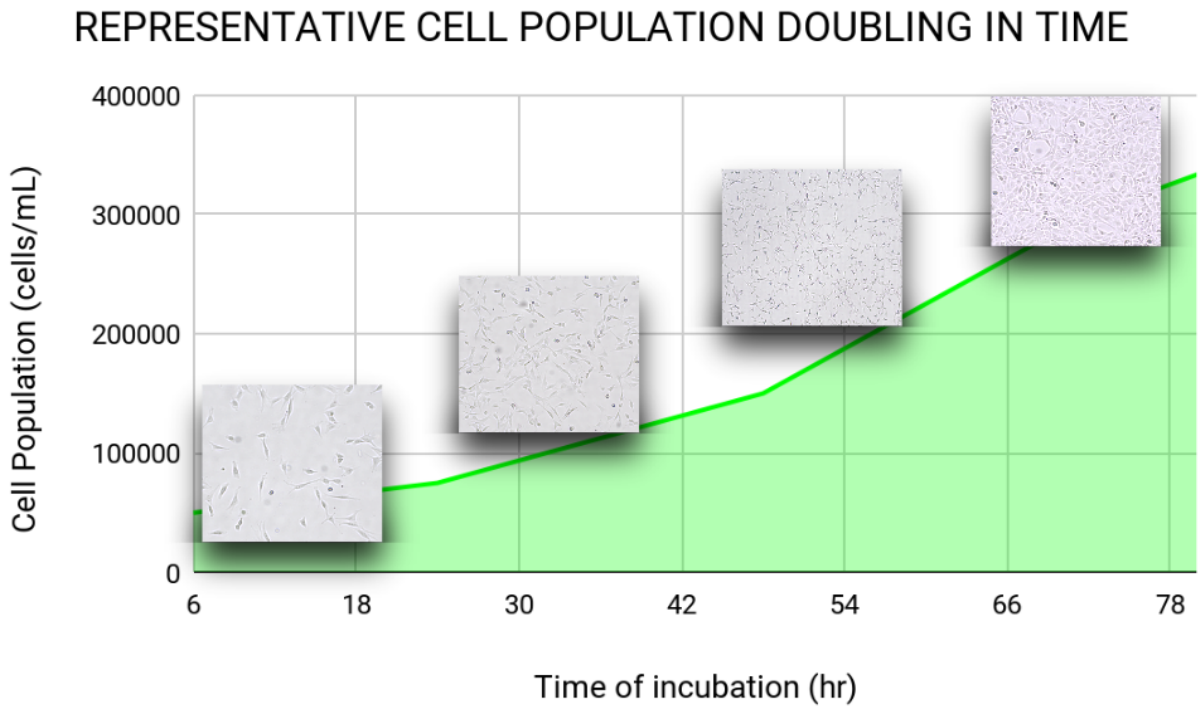

Figure 4: Graph representing NIH-3T3 cells proliferation in time, when cultured with Human FGF2. Population doubling time was determined to be ~20 hours, which is in line with literature.

1.3.2 Fluorescent Cell Images

In addition to the above images, we also highlight intact nuclei (DAPI stained) and actin filament structures (phalloidin stained) to demonstrate the cell boundaries and expected morphology of healthy cells that are growing in media rich with Future Fields' Human FGF2 (Fig. 5).

Fig. 5 - Fluorescent Cell Images

(1) (2) (3)

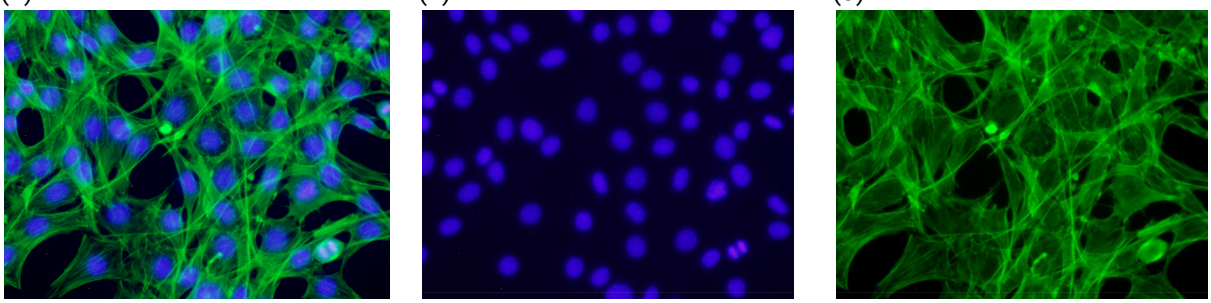

Figure 5: (1) Representative merged images of DAPI and phalloidin stained NIH-3T3 cells in active state of proliferation and growth. (2) DAPI staining indicates intact nuclei structures. (3) Phalloidin staining indicates actin filaments, showing cell boundaries and demarcations.

2. Purity

We purify our growth factors in a proprietary column-based purification strategy (Fig. 6) that helps maintain the structure and functionality of the product throughout the process, while also ensuring that no or limited host components are part of the final products. In Fig. 6, we show **purity > 95 %** of Future Fields' Human FGF2 from different lots in comparison to industry standard.

Fig. 6 - SDS-PAGE Gel Showing Purity of Product

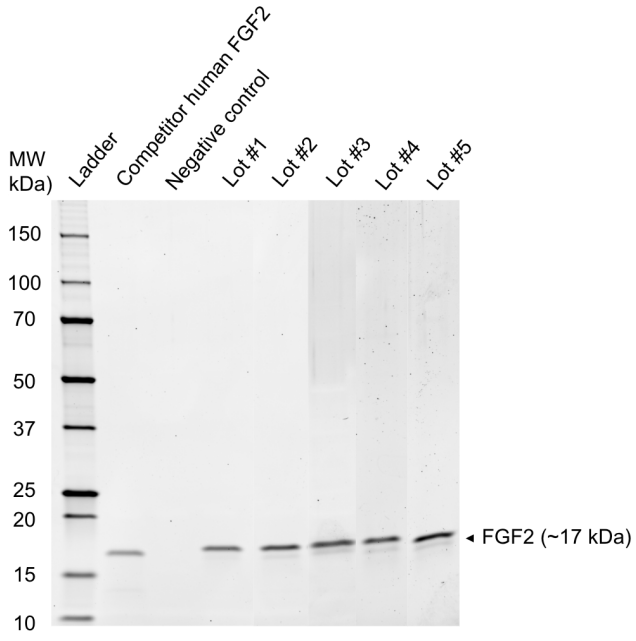

Figure 6: Representative image of SDS-PAGE showing different lots of Future Fields' FGF2 in comparison to competitor standard; proteins run at expected size and no contaminant proteins are visible indicating high purity proteins.

3. Safety

We understand safety is paramount when it comes to cell culture studies. Therefore, we performed assays for the two common contaminants in cell culture media.

3.1 Mycoplasma

A primary concern is *Mycoplasma*, the intracellular antibiotic-resistant contaminant that can have numerous effects on mammalian cell culture. Reassuringly, our proteins are always tested for several species of *Mycoplasma*, *Acholeplasma,* and *Ureaplasma* using a sensitive PCR-based test (Fig. 7) that can detect 1–5 fg of *Mycoplasma* DNA corresponding to 2–5 *Mycoplasma* per sample volume; this ensures we send products that are **negative** for these contaminations.

Fig. 7 - PCR Gel of Mycoplasma Test Results

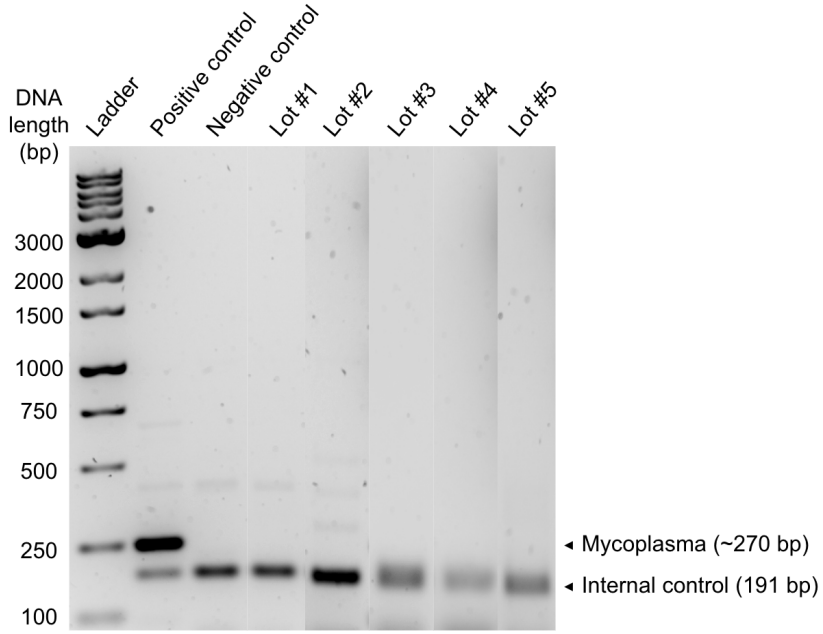

Figure 7: Representative PCR gel image showing positive control for mycoplasma band at 250 bp, and internal control at 191 bp, and negative mycoplasma bands for all lots tested indicating no mycoplasma contamination.

3.2 Endotoxin

Our approach to quality includes rigorous testing for endotoxin, a common name for Lipopolysaccharides (LPS), derived from bacteria that can trigger apoptotic pathways in mammalian cell cultures. Future Fields' Human FGF2 has consistently shown (Fig. 8) to be **below the threshold of FBS (<1 EU/ug)**, thereby reducing any fears of unwanted deleterious cell signaling and effects.

Fig. 8 - Lot-to-Lot Endotoxin Test Results

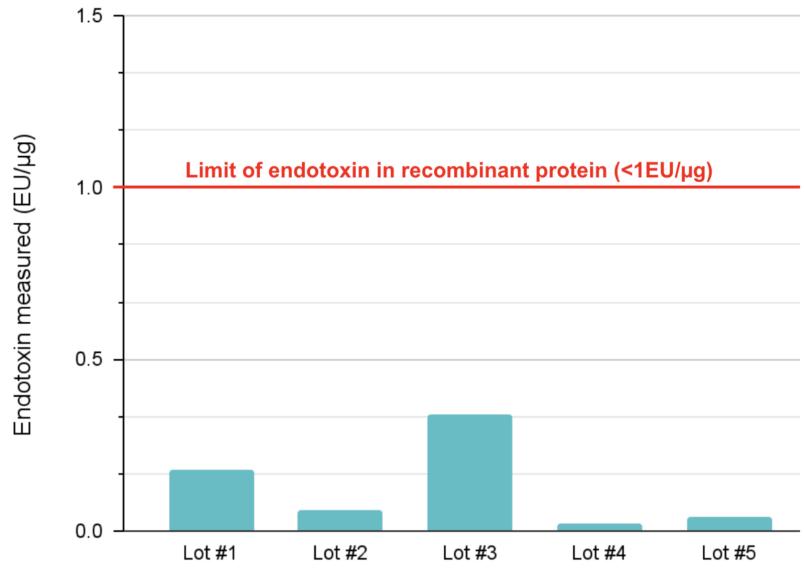

Figure 8: Endotoxin was measured using Chromogenic assay, and all lots tested showed below established threshold values for endotoxin levels indicating safety for mammalian cell cultures.

In conclusion, the above characteristics of Future Fields' Recombinant Human 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 (Fig. 9).

Fig. 9 - ACT Labels for Future Fields' Recombinant Bovine FGF2

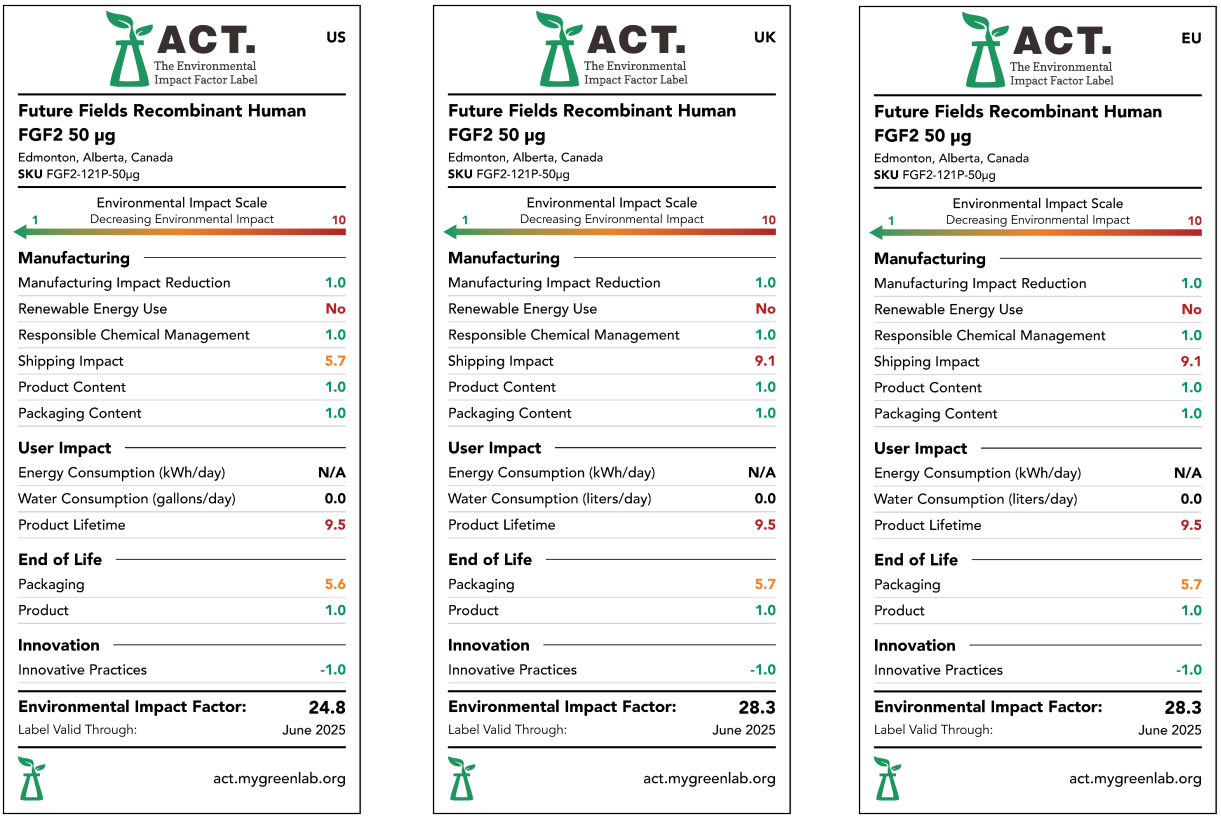

Figure 9: ACT labels depicting the Environmental Impact Factor scores for Future Fields' human FGF2 for US, UK and EU shipments. The Environmental Impact Factor score is 24.8 for US deliveries and 28.3 for UK and EU deliveries. The score does not vary between 50 µg and 100 µg concentrations due to the 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.