Future Fields' Recombinant Human Prolactin, Fc Tag

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.

Lactogen hormones, such as recombinant human prolactin, are critical ingredients for the production of complex products like cultivated milk and potential treatment for lactation insufficiency in the clinic.[1,2] Recombinant human prolactin is a sensitive protein that requires rigorous testing to ensure its functionality.

In this piece, we lay out an in-depth performance analysis of our latest product, **Recombinant Human Prolactin, Fc tag (PRL-Fc)**, to demonstrate its bioactivity, purity, unique features, and adherence to safety standards.

Here is the short version:

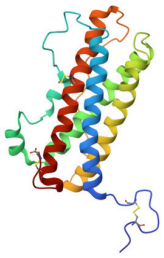
1. **Overview of Human Prolactin:** A pleiotropic hormone
2. **Performance:** Consistent EC_{50} ~ 10-30 ng/ml in cell proliferation assay
3. **Purity:** >90% purity confirmed by SDS-PAGE
4. **Glycosylation:** Preliminary analyses of N-glycosylated PRL-Fc
5. **Safety:**
   - 5.1. Negative for *Mycoplasma* contamination
   - 5.2. Below safety threshold (< 1 EU/µg) for endotoxin
6. **Final Notes:** Designing with quality, affordability, and sustainability in mind
   - 6.1. Bibliography

11130 105 Avenue NW
Edmonton, AB Canada T5H 0L5
support@futurefields.io

1 Overview of Human Prolactin (PRL): A Pleiotropic Hormone

Human Prolactin, or PRL, a hormone synthesized by the pituitary gland, predominantly facilitates lactation in mammary glands, exhibiting pleiotropic effects in both genders.[3-5] Beyond its role in lactation, peripheral Prolactin production correlates with the development of breast and prostate cancer, the regulation of reproductive function, and immune system modulation. Recent investigations propose cytoprotective attributes of PRL on pancreatic islet cells and liver tissue, presenting potential significance in transplantation settings.[6,7]

Figure 1 - Ribbon Structure of Human Prolactin (PRL)



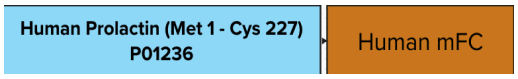
The two prevailing forms of Little Prolactin are glycosylated and non-glycosylated forms, with a molecular weight of ~23 kDa. In physiology, PRL manifests in dynamic ratios of glycosylated and non-glycosylated forms, contingent upon various factors.

Moreover, post-translational modifications dictate their affinity to receptors and in vivo half-life. Glycosylated PRL is demonstrated to have diminished receptor affinity but an extended half-life compared to non-glycosylated forms.[4,8-10]

When prolactin binds to the prolactin receptors (PRL-R), it triggers dimerization of receptors. This results in the activation of Janus kinase 2 (Jak2), a tyrosine kinase that initiates the JAK-STAT pathway. Interestingly, heat shock proteins are also expressed upon activation via the PRL-R, resulting in demonstrated cytoprotective effects on liver and pancreatic cells.[11]

Future Fields' Recombinant Human Prolactin, Fc tag (PRL-Fc) carries a human monomeric IgG1 Fc tag at the C-terminus. This product is sold as lyophilized for ease of use and extended storage.

Figure 2 - Human Prolactin, Fc Tag Sequence

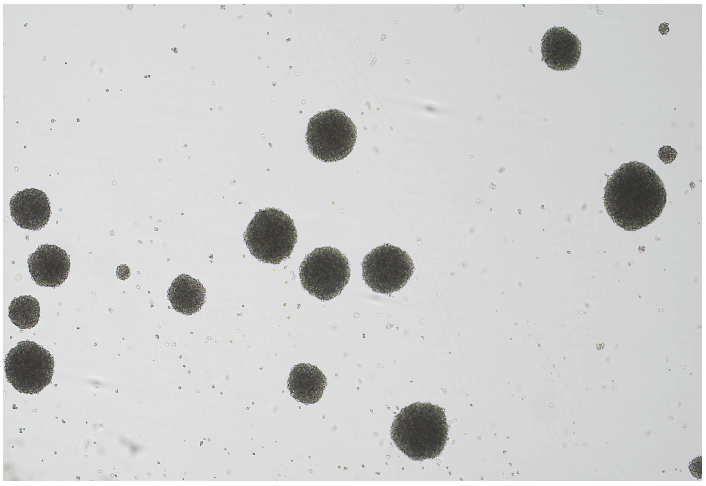


2 Performance

To characterize Future Fields' PRL-Fc for its functionality, we use proliferation assay in NB2-11 cells (pre-T rat lymphoma that is dependent on PRL for mitogenesis).[12] Using a metabolic activity-based readout, we determine that PRL-Fc is able to drive proliferation of cells in a dose-dependent manner (EC_{50} ~ 10-30 ng/ml), and is highly comparable to existing industry offerings (Figure 3). The EC_{50} is defined as the effective concentration of the growth factor at which cell proliferation is at 50% of maximum.

Figure 3 - PRL-Fc Functionality Assessment with Cell Proliferation Assay

A)



B)

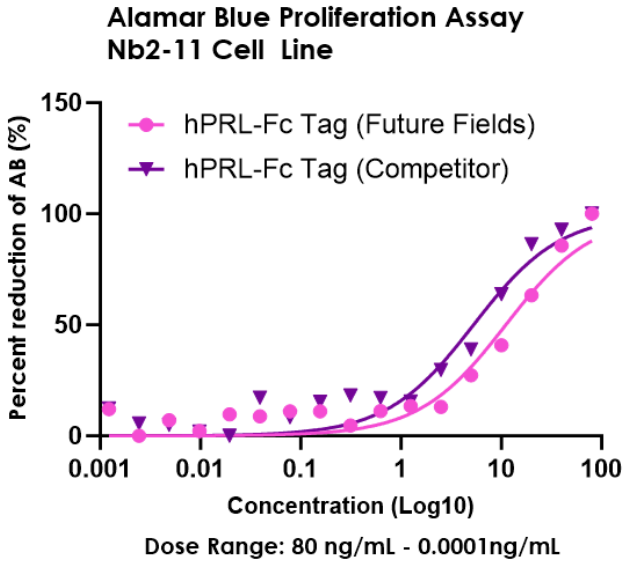


Figure 3: A) Representative brightfield image of healthy, proliferating NB2-11 cell lines, displaying classic colonies in suspension media. B) Functionality of PRL-Fc was determined by its ability to induce the proliferation of rat NB2-11 cells in a dose dependent manner (EC_{50} ~ 10-30 ng/ml).

3 Purity

We purify our growth factors in a proprietary column-based purification technique. In Figure 4, we show purity >90% of multiple Lots of Future Fields' PRL-Fc, in comparison to an existing industry standard. The protein migrates at 52 kDa under reducing (R) condition (SDS-PAGE).

Figure 4 - Lot-to-Lot Consistency of High-Purity PRL-Fc

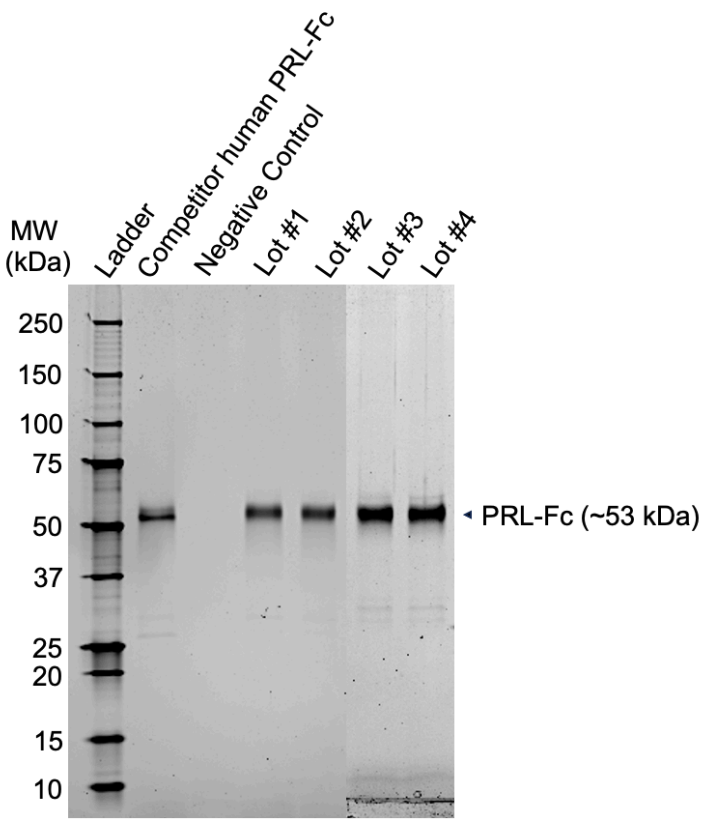


Figure 4: Representative image showing SDS PAGE gel with multiple Lots of PRL-Fc in comparison to an existing industry standard.

4 Glycosylation Analysis of PRL-Fc

To reveal the nature of N-glycosylation of PRL-Fc, we used deglycosylation enzyme treatment. PNGase F was employed for the enzymatic N-deglycosylation of PRL-Fc expressed in *Drosophila melanogaster.* Peptide:N-glycosidase F (PNGase), which cleaves the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins and glycopeptides, was able to cleave off glycans presented on the protein. This results in a discernible shift in the molecular weight of the protein and runs lower than the glycosylated form of PRL, as seen by Sypro stained gel (Figure 5) and Western blot probed with anti-PRL antibody (Figure 6).

The below data indicates that PRL-Fc produced using the EntoEngine™ platform possess N-glycosylation post-translational modifications that emulates physiological nature of the protein. Further analyses of the N-glycosylation pattern is ongoing and will reveal its impact on stability and functionality on the protein.

Figure 5 - Sypro Stained SDS Gel

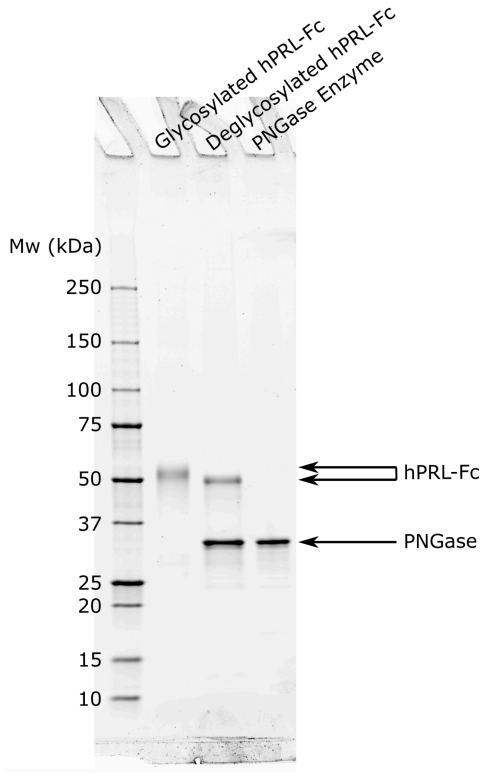


Figure 5: Sypro stained SDS gel showing a shift in molecular weight for PRL-Fc after PNGase treatment.

Figure 6 - Western Blot Analysis

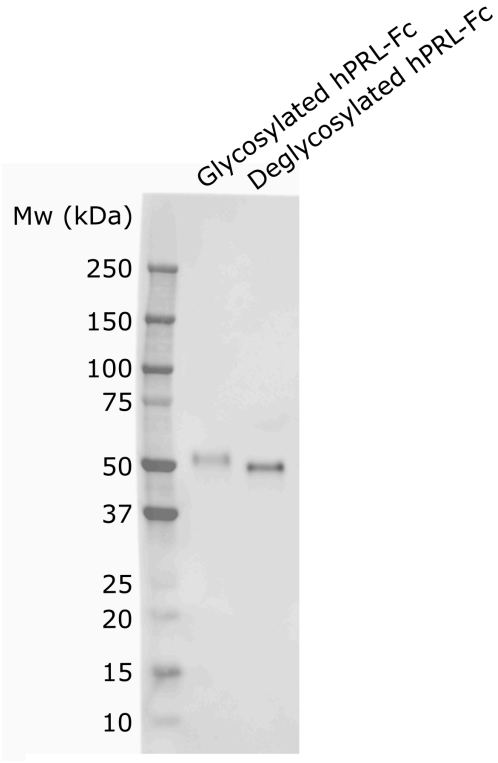


Figure 6: The Western blot of PRL-Fc probed with anti-PRL antibody (sc-46698) reveals a discernible shift in molecular weight for PRL-Fc, post-treatment with PNGase.

5 Safety

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

5.1 *Mycoplasma*

A primary concern in cell cultures is *Mycoplasma*, the intracellular antibiotic-resistant contaminant that can have numerous effects on mammalian cells. Reassuringly, our proteins are tested for several species of *Mycoplasma, Acholeplasma*, and *Ureaplasma* using a sensitive PCR-based test (Figure 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.

Figure 7 - Gel Showing PCR Products of *Mycoplasma* Test

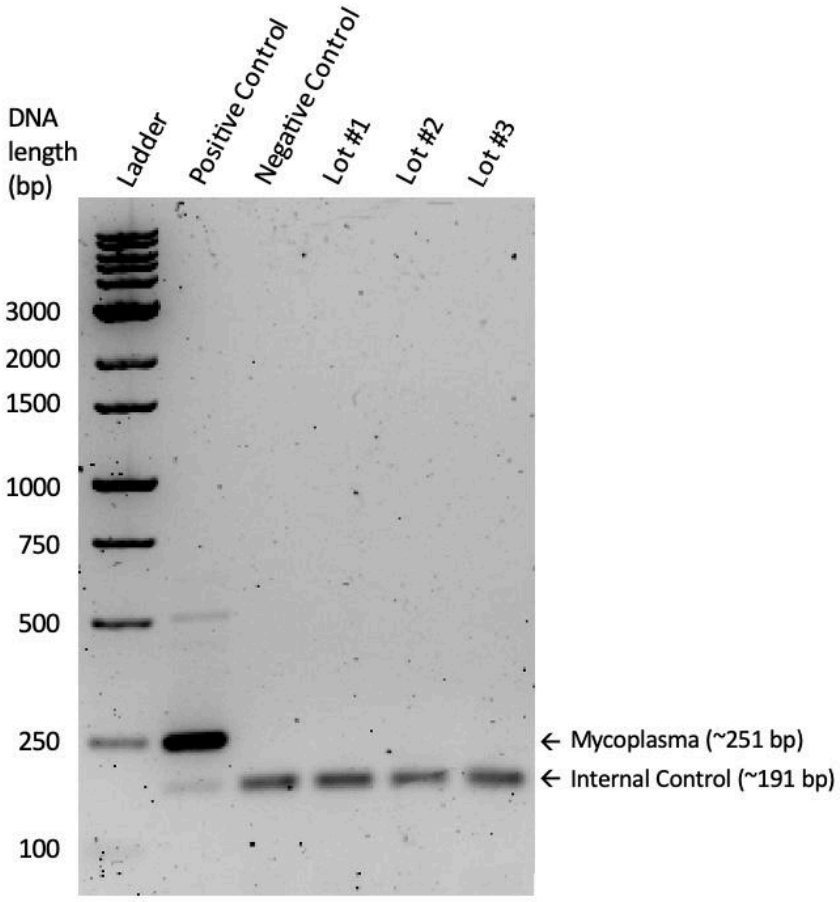


Figure 7: Representative PCR gel image showing positive control for Mycoplasma band at 250 bp, and internal control at 191 bp, and negative Mycoplasma bands for all Lots tested indicating no Mycoplasma contamination.

5.2 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' PRL-Fc have consistently shown (Figure 8) to be below the threshold of FBS (<1 EU/µg), thereby reducing any fears of unwanted deleterious cell signaling and effects.

Figure 8 - Lot-to-Lot Endotoxin Test Results

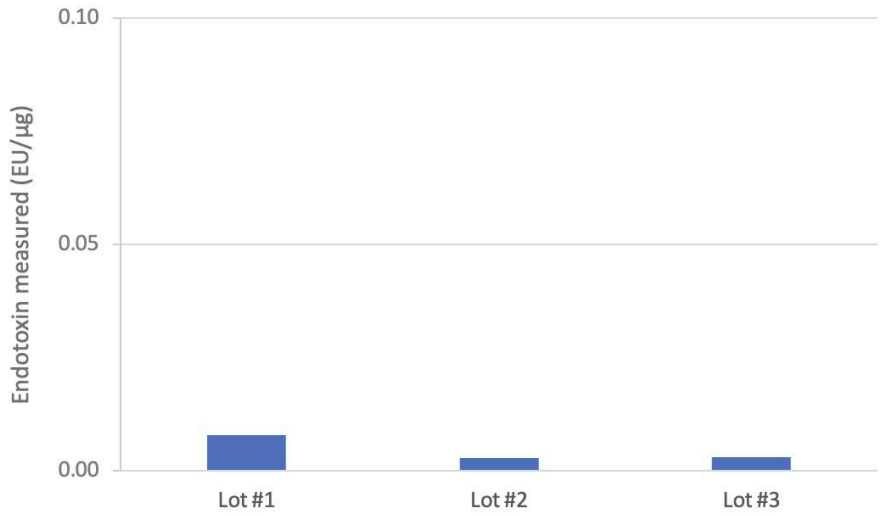


Figure 8: Endotoxin was measured using Chromogenic assay, and all Lots tested showed below established threshold values for endotoxin levels indicating safety for mammalian cell cultures. Limit of endotoxin in recombinant protein (< 1 EU/µg).

In conclusion, the above characteristics of Future Fields' Recombinant Human PRL-Fc produced using EntoEngine™ can meet your expectations for consistency of high performance and safety to support your research and production goals.

6 Designing Proteins with Quality, Affordability, and Sustainability in Mind

Our performance report is a testament to our dedication to provide our customers with a high quality product that fits seamlessly with their experiments and protocols. Our robust quality team conducts rigorous assessments to ensure Lot-to-Lot excellence.

At the same time, our fruit fly-based EntoEngine™ platform produces our complex recombinant proteins at scale. The cost-efficiency of using *Drosophila melanogaster* as the expression system is why we can sell our proteins at a fraction of the cost of market price today, without compromising quality.

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

Get started by requesting a sample today.

6.1 Bibliography

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