Future Fields' Recombinant Human Prolactin (Tag-Free)

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 (Tag-Free)**, 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} ~ 1-3 ng/ml in cell proliferation assay
3. **Purity and Identity:** >85% purity confirmed by SDS-PAGE
4. **Glycosylation:** PNGase F analyses of N-glycosylated PRL
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

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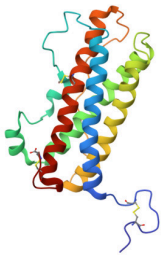
1 Overview of Human Prolactin (PRL): A Pleiotropic Hormone

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]

We understand that, in some instances, having a tag in the final product can create complications both from application and regulatory requirements perspectives. We listened and are delighted to offer a tag-free version of PRL for your benefit.

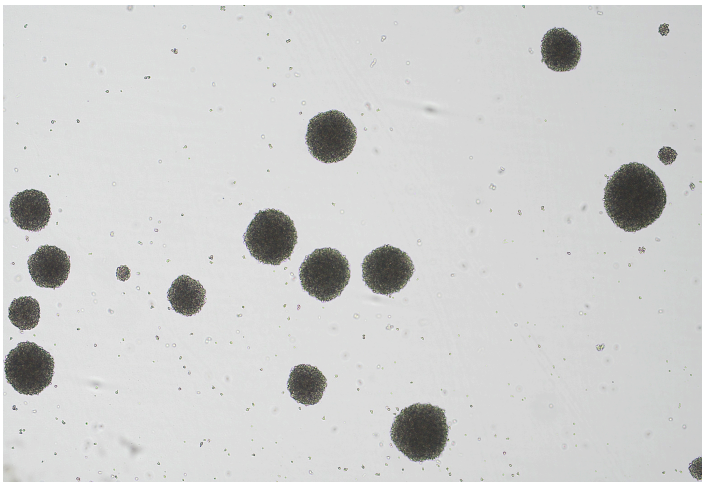
Figure 2 - Human Prolactin (Tag-free)

2 Performance

To characterize Future Fields' PRL (tag-free) for its functionality, we use proliferation assays in NB2-11 cells (pre-T rat lymphoma that is dependent on PRL (tag-free) for mitogenesis).[12] Using a metabolic activity-based readout, we determine that PRL (tag-free) is able to drive proliferation of cells in a dose-dependent manner (EC_{50} ~ 1-3 ng/ml) (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 (Tag-Free) Functionality Assessment with Cell Proliferation Assay

A)



B)

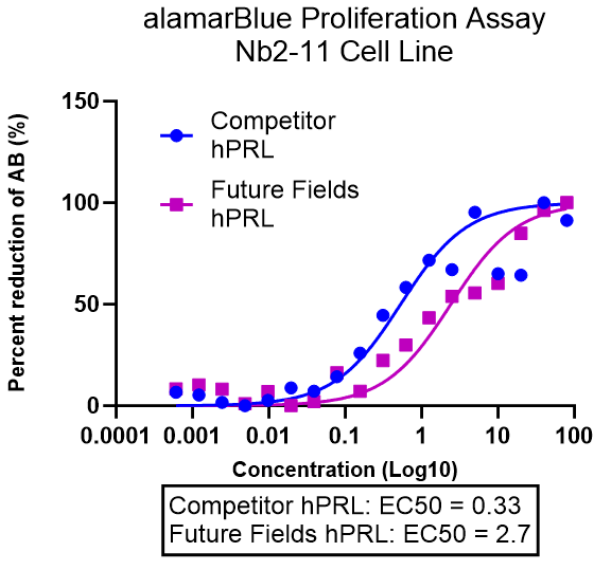


Figure 3: A) Representative brightfield image of healthy, proliferating NB2-11 cell lines, displaying classic colonies in suspension media. B) Functionality of PRL (tag-free) was determined by its ability to induce the proliferation of rat NB2-11 cells in a dose dependent manner (EC_{50} ~ 1-3 ng/ml).

3 Purity and Identity

We purify our growth factors in a proprietary column-based purification technique. In Figure 4A, we show purity >85% of Future Fields' PRL (tag-free) in comparison to an existing industry standard. The protein migrates at ~26 kDa under reducing (R) condition (SDS-PAGE) and displays two bands, indicating the presence of glycosylated and non-glycosylated forms of the protein. Furthermore, the 16 kDa faint band was determined to be truncated PRL based on mass spec results. More data is available upon request.

Identity of PRL (tag-free) was confirmed using western blotting with anti-PRL antibody (Figure 4B). Western blot analyses of Future Fields PRL (tag-free) shows two bands, corresponding to glycosylated and non-glycosylated forms of the protein, and can be seen in comparison to the non-glycosylated *E. coli* derived industry standard.

Figure 4 - Purity (SDS PAGE) and Identity of High-Purity Glycosylated PRL (Tag-Free)

A) B)

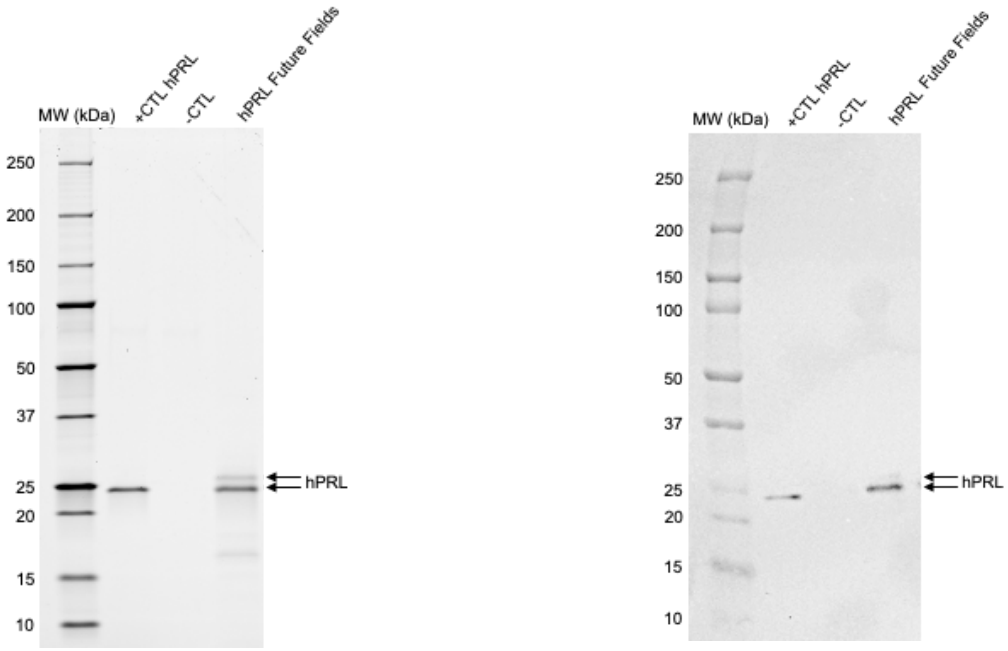


Figure 4: A) Representative image showing SDS PAGE gel with PRL (tag-free) in comparison to an existing industry standard. B) Representative western blot showing PRL (tag-free) in comparison with E.coli derived commercial standard, probed with anti-PRL antibody.

4 Glycosylation Analysis of PRL (Tag-Free)

To reveal the nature of N-glycosylation of PRL (tag-free), we used deglycosylation enzyme treatment. PNGase F was employed for the enzymatic N-deglycosylation of PRL (tag-free) expressed in *Drosophila melanogaster*. Peptide: N-glycosidase F (PNGase F)–which cleaves between 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).

The below data indicates that PRL (tag-free) 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 Showing N-glycosylation Analysis

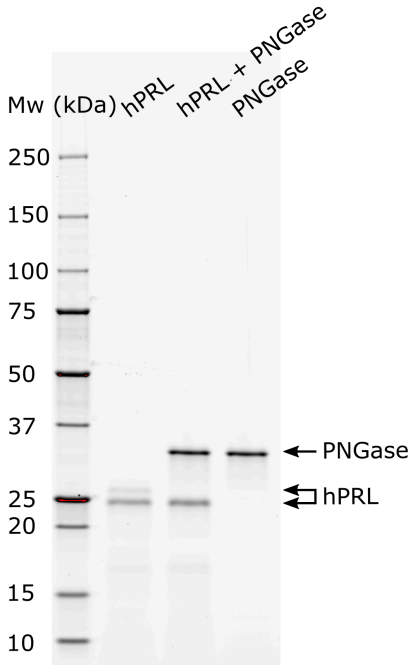


Figure 5: Sypro stained SDS gel showing a shift in molecular weight for PRL (tag-free) after PNGase F treatment indicating the presence of N-glycosylated PRL in the final product. About 20% of our product is in N-glycosylated form.

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 using a qPCR-based test validated by the manufacturer to meet the requirements of European Pharmacopoeia (EP) 2.6.7 in combination with EP 2.6.21. The test has a detection limit of less than 10 CFU/mL for all *Mycoplasma* species mentioned in the EP and fulfills the requirements for sensitivity, robustness and specificity for cell cultures and autologous cell transplants. This ensures we send products that are negative for these contaminations.

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. Every lot of Future Fields' PRL (tag-free) is tested using methods compliant with USP <85> and must be below the threshold of FBS (<1 EU/µg), thereby reducing any fears of unwanted deleterious cell signaling and effects.

In conclusion, the above characteristics of Future Fields' Recombinant Human PRL (tag-free) 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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