

BIOACTIVE AND PROPERLY FOLDED PROLACTIN IS OBTAINED IN *E. COLI* IN A PILOT BIOREACTOR AT 37°C BY CONSTITUTIVE EXPRESSION BASED ON THE UNREPPRESSED λ P_L PROMOTER

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Human prolactin (hPRL) is a polypeptide hormone of molecular mass 23,000 with a single chain of 199 residues and three disulfide-bonded loops. It is one of the most versatile hormones in terms of biological actions, more than 100 different effects having been documented. Our research group has been the first to obtain useful secretions of hPRL in *E. coli* periplasmic space but, differently from human growth hormone (hGH), the utilization of a temperature controlled λ P_L promoter only provided extremely low amounts of the hormone (~0.03 μ g/mL/A₆₀₀). We describe the set up, in shake flask cultures, of a constitutive hPRL expression based on the unrepressed λ P_L promoter, under optimized temperature conditions (37°C). This way useful specific expression levels of about 0.9 μ g hPRL/mL/A₆₀₀ were obtained. Since no industrial production of hPRL in *E. coli* has ever been described, we also set up pilot scale cultivation conditions that provided a useful specific expression of 1.0 μ g hPRL/mL/A₆₀₀ at an optical density of 35 A₆₀₀. Its purification and characterization confirmed that the protein is highly monomeric, authentic and properly folded, with a biological activity of ~51 IU/mg, not significantly different from the first WHO Reference Reagent (97/714).

Key words: *E.coli*, fed batch fermentation, human prolactin, λ P_L promoter

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