Transient expression of recombinant human prolactin and thyrotropin in human embryonic kidney (Expi293F™) suspension cells

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Human prolactin (hPRL) and human thyrotropin (hTSH) are pituitary polypeptide hormones with key functions in the physiological regulation of the human body. hPRL is highly secreted during lactation, has important action in reproduction and for immunoregulation, among other functions. hTSH is related to the control of thyroid gland. The Chinese Hamster Ovary (CHO) and Human Embryonic Kidney (HEK293) cells are the most used hosts for expression of recombinant human proteins because they can be easily cultured in suspension conditions, and express high levels of proteins that have a relative similarity in post-translational modifications compared to their human counterparts. Our laboratory has experience in the synthesis of these proteins in the *Escherichia coli* periplasm (hPRL), adhered CHO (hPRL and hTSH), suspension CHO (hPRL) and adhered HEK293T cells (hTSH). The aim of this work was to produce hPRL and hTSH in suspension Expi293F™ cells for their characterization. The hPRL and hTSH cDNA were introduced into the commercial plasmid pcDNA™ 3.4-TOPO® and 30 µg of these plasmids were used to transfect 30 mL of suspension Expi293F™ cells (2.5 x 10^6 cells/mL) in a 125 mL erlenmeyer, using 81 µL of ExpiFectamine™ transfection agent. After 16 h of transfection, 150 µL of Enhancer 1 and 1.5 mL of Enhancer 2 were added and the culture was maintained in an incubator at 37 °C, 8% CO_2, at 125 rpm in orbital shaker. Samples of conditioned media (Expi293™ expression medium) were collected during 4 days and stored at -80 °C. These were analyzed by SDS-PAGE, ELISA, Western blotting, and HPLC. For the first time, hPRL and hTSH, were transiently expressed in human (Expi293F™) suspension cells, the expression levels reaching, on the 3rd day, 46 µg of hPRL/mL and 116 µg of hTSH/mL. These results show that the expression is clearly dependent on the characteristics of the protein and that this methodology is very efficient to obtain high levels of human glycoproteins in a short time and will allow us to purify them and compare their glycosylation profiles of these to CHO-derived and human native pituitary hormones.