

## ANALYSIS OF HUMAN THYROID STIMULATING HORMONE (hTSH) OBTAINED IN CHO CELLS UNDER DIFFERENT CULTURE CONDITIONS

*Oliveira, JE; Loureiro, RF; Peroni, CN; Bartolini, P, Ribela, MTCP  
IPEN/CNEN-SP, São Paulo – Brasil - Biotechnology Department - CBM (mtribela@ipen.br)*

Culture parameters have been recognized as critical factors influencing the expression level of a protein. In this work we examined the effects, on the production of recombinant human thyrotropin (rec-hTSH), of two conditions that have been reported to enhance the production of certain proteins in CHO cells: level of carbon dioxide (CO<sub>2</sub>) and temperature. Rec-hTSH, a human glycoprotein hormone, was produced by CHO cells according two schemes: one of them was considering an atmosphere with 5% CO<sub>2</sub> and the other no CO<sub>2</sub> supply. The level and the quality of hTSH secreted into the culture medium were determined. No significant difference was observed concerning total TSH production, although some differences regarding the kinetics of hormone secretion could be noted. Utilizing a strategy according to which the cells are grown at 37°C until reaching confluence, followed by a temperature shift to 30°C for the remaining time versus the classical strategy of maintaining the cells at 37°C during all the culture period, no significant differences in the level and in the quality of hTSH secreted into the culture medium were observed. Considering that differences in cell culturing conditions during glycoproteins synthesis can potentially alter glycosylation pattern and consequently their biological activities, we are also carrying out the carbohydrate compositional analysis of the different hTSH preparations, together with a qualitative RP-HPLC and HPSEC analysis that follows a previously set up methodology (Oliveira JE et al., J. Chromatogr. B 2003; 787:345-355).

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