

## PROTEIN ENGINEERING TO ENHANCE BIOPROCESSING

### PC 73. EXPRESSION OF THE HUMAN PROLACTIN ANTAGONIST DELTA 1-11 G129R-PRL IN *E. COLI* PERIPLASM

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Recombinant human prolactin antagonist delta 1-11 G129R-hPRL is a 21.9 kDa protein with 188 amino acids that downregulates the proliferation of a variety of cells expressing prolactin receptors. Periplasmic expression of recombinant proteins in *E. coli* has been considered an option for obtaining an authentic, soluble, and correctly folded protein, as an alternative to the cytoplasmic production in inclusion bodies of an unfolded, insoluble protein, carrying an extra initial methionine. The aim of this work was to carry out the expression of delta 1-11 G129R-hPRL antagonist in the periplasm of *E. coli*, testing different temperatures. *E. coli* BL21(DE) strain, transformed with a plasmid based on a pET25b(+) vector, DsbA signal peptide and delta 1-11 G129R-hPRL cDNA, was cultured in LB medium with ampicillin addition. After overnight culture at 30 °C, 0.6 mM IPTG was added and five different temperatures were applied: 25, 30, 32, 35 and 37 °C. Periplasmic fluid was extracted after 5 hours by osmotic shock. The samples were analyzed by SDS-PAGE, Western blotting and RP-HPLC. The best condition was increasing the temperature to 35 °C for 5 h, after having reached the late log phase. The specific expression of  $0.14 \pm 0.02 \mu\text{g/mL/A}_{600}$ , with a final optical density of  $3.43 \pm 0.13 A_{600}$  ( $n = 3$ ) was obtained. Purification by nickel affinity chromatography (Hisprep FF) with Imidazole elution followed by size exclusion chromatography (Sephacryl S-100) was carried out connected to an ÄKTA purification system. Quantification was carried out by comparison between the areas under the curve observed in the HPSEC chromatogram, for the unknown samples versus the Internal Standard of rec-hPRL. The final product showed >95% purity by HPSEC analysis. The delta 1-11 G129R-hPRL antagonist was expressed and purified for further *in vivo* and *in vitro* tests, in view of clinical applications for inhibiting cancer cell proliferation that overexpresses prolactin receptor and studies related to prolactin function in anterior pituitary.

**Keywords:** recombinant antagonist of prolactin, periplasmic expression, DsbA signal peptide.

**Development Agency:** Instituto de Pesquisas Energéticas e Nucleares, IPEN – CNEN/SP.