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ISOLATION AND PURIFICATION OF BIOSYNTHETIC HUMAN PROLACTIN EXPRESSED IN THE ESCHERICHIA COLI PERIPLASMIC SPACE

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Recombinant, fully bioactive authentic human prolactin (hPRL) has been synthesized in transformed *Escherichia coli* in a soluble, monomeric, non glycosylated form, which is secreted into the bacterial periplasm. Extraction of the protein was carried out through a modified osmotic shock procedure. Reverse-phase-HPLC (RP-HPLC) was used for characterization and quantification of the hormone in the crude extract released from the periplasmic fluid. Different osmotic shock protocols were also tested to improve the recovery of the hPRL.

A two-step purification process was used. The osmotic shock extract was applied onto a cation exchange column (SP-Sepharose Fast Flow) followed by a size exclusion chromatography (Sephacryl S-100). The final product presented a purity higher than 97%, with a final yield of approximately 50%.

Analysis of the product obtained was performed by SDS-PAGE, Western Blotting, radioimmunoassay, RP-HPLC and size exclusion HPLC. The biological activity was tested through the proliferative assay on rat Nb2 lymphoma cells.

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