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A NOVEL, HIGH YIELD PURIFICATION OF RECOMBINANT AUTHENTIC HUMAN PROLACTIN HORMONE

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The human prolactin hormone (hPRL) role in several diseases and imunological pathways has been thoroughly investigated in the last decade. Despite presenting a wide range of biological activities, the most interesting and useful features of this hormone would be those related to its immunomodutalor function which has been linked to pathologic states as cystic fibrosis, systemic lupus erythematosus, acute allergic encephalomyelitis, rheumatoid arthritis, adjuvant arthritis and tumor growth. Another remarkable biological activitie would be that described for a cleaved isoform of hPRL that showed antiangiogenic properties *in vitro*. Thus the avaliability of hPRL as a biological active protein turns out to be an important issue on the development of further studies about its functions and relationships among a multitude of research options. The present work describes the production and isolation of recombinant authentic hPRL in transformed *E.coli* as well as a novel, high yield, 2-step purification process making use of a hPRL metal binding feature recently described. The purification basically consists of a initial immobilized metal affinity chromatography (IMAC) using Ni^{2+}as the affinity metal compound, in which we readily obtain a highly purified hPRL (96%) followed by a size exclusion column as a polishing and desalting step yielding a monomeric , bioactive (32.0 i.u./mg) hPRL with a purity of 98 % and an overall recovery of 75%.

The purity throughout the process was tested by SDS-PAGE, Bradford protein determination, reverse phase high performance chromatography (RP-HPLC) and radioimmuneassay (RIA). The further characterization of the hPRL obtained was performed by Western blotting, SDS-PAGE, RP-HPLC, size exclusion high performance chromatography (SE-HPLC), isolelectric focusing, aminoacid sequencing, Nb2 bioassay and mass spectrometry.

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