

Highly Efficient Extracellular Production of Human β -Endorphin in Recombinant *Escherichia coli* by Using Outer Membrane Protein F as a Fusion Partner

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Human β -endorphin, producing in human a good feeling and tolerance to pain, is hundreds or even thousands of times more potent than morphine on a molar basis. For convenient recovery of this polypeptide, an efficient extracellular production system was constructed with *Escherichia coli* by using outer membrane protein F (OmpF) as the fusion partner. In details, the ompF gene of *E. coli* BL21(DE3) was first knocked out by using the red operon of bacteriophage λ to construct *E. coli* MBEL-BL101. The β -endorphin gene was fused to the C terminus of the *E. coli* ompF gene by using a linker containing the Factor Xa recognition site. A novel fed-batch cultivation strategy was developed in pilot bioreactor for the high-level production of OmpF β -endorphin fusion protein. Then, the fusion protein in the culture medium was concentrated and purified by anion-exchange chromatography and cleaved by Factor Xa to yield β -endorphin, which was finally purified by reverse-phase chromatography. In conclusion, a simple and efficient production process for β -endorphin production has been established, taking into account upstream to downstream processing. Furthermore, this efficient expression strategy is po-

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