

## عنوان مقاله:

Expression and Purification of Soluble form of Human Parathyroid Hormone (rhPTH<sub>1-34</sub>) by Trx Tag in E. coli

## محل انتشار:

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## خلاصه مقاله:

Background: Parathyroid Hormone (PTH) is secreted by parathyroid glands and controls the level of calcium in bones and kidney. PTH is a small polypeptide with 84 amino acids, but the first 34 amino acids of which are enough for hormone biological activity and can be used in the treatment of Osteoporosis. The expression efficiency of recombinant human parathyroid hormone rhPTH (1-34) or Teriparatide using a cleavable fusion protein strategy was compared in two strains of E. coli. Materials and Methods: A cassette was designed and fully synthesized for prokaryotic expression of rhPTH using pET system. From 5' to 3', the cassette consisted of: Trx tag to increase the solubility of protein, His tag for purification and detection of protein, enterokinase site to cleave all fusion moieties, and an optimized gene code for Teriparatide corresponding to the amino acid sequence of hPTH. This cassette was cloned into pET32a vector. The vector was simultaneously transformed and expressed in two different E. coli strains. The ability of strains for expression of this recombinant pharmaceutical was compared. Early expression was confirmed by SDS-PAGE and Western Blotting. The soluble fusion protein was harvested and purified by immobilized affinity chromatography. Then the fusion moiety was released from Teriparatide by enterokinase digestion. Results: The fusion form of rhPTH was efficiently expressed in both E. coli strains. However, the percentage of the target protein to the total protein content in Rosetta-gami was more than its amount in BL21 (60 % vs 25%). The fusion protein was highly purified with Ni-NTA column. Up to 18.5 mg/ml of pure fusion protein has been obtained from 1-liter Rosetta-gami strain of E. coli. The pure Teriparatide was released by enterokinase digestion. Conclusion: The pure rhPTH (1-34) produced here, could be the subject for biological activity and quality control assessments, and following formulation processing, it could be applied as a peptide drug in the treatment of Osteoporosis.

## کلمات کلیدی:

Enterokinase, Fusion protein, parathyroid hormone, BL21, Rosetta-gami

