

## The best pH of elution buffer for purification of recombinant fusion Teriparatide with Ni<sup>2+</sup> affinity chromatography in denaturing condition

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### Abstract

The first 34 amino acids of parathyroid hormone has complete activity of 84 amino acids parathormone and is produced as recombinant form, teriparatide. This recombinant peptide drug increases bone density leads to fracture risk reduction. This drug has been used for treatment of osteoporosis in women and men. A new 115 amino acids teriparatide fusion protein was purified from total protein of host cell with elution buffer in different pHs.

**Introduction:** Proteins are widely used in pharmacology, medicine, diagnostics and researches. *Escherichia coli* is one of the most frequently used hosts for recombinant drug production. Consequently, purification of recombinant protein expressed in this host is very important. This study was done to find the best pH of elution buffer for purification of recombinant teriparatide fusion protein expressed in *E.coli* BL21 (DE3). For this purpose, elution stage was performed in 3 different pHs.

**Methods and Results:** Teriparatide fusion protein was purified with nickel affinity chromatography in denaturing buffers with different pHs. There are 3 specified steps in this type of chromatography includes interacting between the fusion protein and the positive nickel ions of the resin, washing the host proteins, and to finish eluting the fusion protein. Non-specific proteins were eliminated from the column with pH 6.3 washing buffer [100mM NaH<sub>2</sub>PO<sub>4</sub>, 10mM Tris buffer, 8M Urea, 20mM 2-Mercaptoethanol] and recombinant fusion teriparatide has the His-tag was eluted via elution buffers [as same as washing buffer] in 3 states with 3 pHs of 5.8, 4.6 and 4.2. The micro Bradford test was done in all mentioned stages. As a final point, the samples were flow thrown, washed and eluted observed in SDS-PAGE.

**Conclusions:** Six histidin residues in N-terminal of fusion teriparatide sequence were interacted easily with the positively charged nickel ions of resin. In this way, the recombinant fusion protein, teriparatide, was hold in the resin at pHs higher than His-tag pI (5.1) and disconnected from the resin at pH slower than it. In this work, major amounts of teriparatide were exited from column at pH 4.6.

**Key words:** Teriparatide, Purification, Affinity Chromatography, Ni<sup>2+</sup> Column