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(54) **Title**: A PROCESS FOR OBTAINING EXENDIN-4

(57) **Abstract**: The present disclosure provides a process for preparing Exendin-4 from high cell density employing specific steps to obtain purified Exendin-4. The process produces Exendin-4 in higher purity and yield, employing chromatographic and non-chromatographic techniques in the process. The present disclosure also provides for a process for purification of Exendin-4.

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1. A process for obtaining Exendin-4, said process comprising acts of:
   a) obtaining vector comprising gene for Exendin-4 set forth in SEQ ID No. 1 and transforming host cell with the vector to obtain transformed cell;
   b) culturing the transformed cell in culture medium and subjecting to fermentation;
   c) lysing the cell to obtain crude Exendin-4 and subjecting cell lysate to clarification to obtain clarified sample;
   d) subjecting the clarified sample to affinity chromatography and tangential flow filtration to obtain protein;
   e) digestion of the protein; and subjecting the digested protein to chromatography and filtration to obtain purified Exendin-4.

2. The process as claimed in claim 1, wherein the gene sequence for the Exendin-4 includes solubility or affinity tag or combination thereof; and the Exendin-4 is expressed as a fusion protein.

3. The process as claimed in claim 1, wherein the host cell is E.coli; and the vector is selected from group comprising pET-32a and pET24.

4. The process as claimed in claim 1, wherein the transforming is carried out by technique selected from group comprising Heat shock method, electroporation and chemical method or any combinations thereof; and the culturing is carried out at temperature ranging from about 25°C to about 40°C, preferably about 37°C, for time duration ranging from about 1 hour to about 20 hours, preferably about 17 hours, at pH ranging from about 6.8 to about 7.4, preferably about 7.2.

5. The process as claimed in claim 1, wherein the fermentation is carried out at temperature ranging from about 35°C to about 40°C, preferably about 37°C, for time duration ranging from about 11 to 13 hours, at pH ranging from about 6.5 to 7.5.

6. The process as claimed in claim 1, wherein the lysing of cell is carried out by subjecting the cell to high pressure Homogenizer or Microfluidizer or French Press, or any combinations thereof; and wherein the clarification of the cell lysate is carried out by techniques selected from group comprising centrifugation, tangential flow filtration and microfiltration; or any combinations thereof.

7. The process as claimed in claim 1, wherein the digestion of protein is with enterokinase enzyme; and the chromatography of step c) is selected from group comprising affinity chromatography and reverse phase chromatography, or combination thereof; and the filtration is selected from group comprising ultrafiltration, diafiltration and sterile filtration, or any combinations thereof.

8. The process as claimed in claim 1, wherein the Exendin – 4 obtained by the process has a purity of at least 97%, preferably about 99%; and the Exendin-4 is formulated into dosage form in formulation buffer comprising component selected from group of sodium acetate, acetic acid, D-Mannitol and Meta-cresol or any combinations thereof.

9. A process of purifying Exendin-4, said method comprising acts of:
    a) lysing cell comprising Exendin-4 protein and subjecting the cell lysate to clarification to obtain clarified sample;
    b) subjecting the clarified sample to affinity chromatography and tangential flow filtration;
    c) digestion of protein; and subjecting the digested protein to chromatography and filtration to obtain purified Exendin-4.

10. The process as claimed in claim 9, wherein the lysing of cell is carried out by subjecting the cell to high pressure Homogenizer or French Press or Microfluidizer, or any combinations thereof; and the clarification of the cell lysate is carried out by techniques selected from group comprising centrifugation, tangential flow filtration and microfiltration; or any combinations thereof.

11. The process as claimed in claim 9, wherein the digestion of protein is with enterokinase enzyme; and the chromatography of step c) is selected from group comprising affinity chromatography and reverse phase chromatography, or

combination thereof; and the filtration is selected from group comprising ultrafiltration, diafiltration and sterile filtration, or any combinations thereof.

12. The process as claimed in claim 9, wherein the protein is fusion protein; and the filtration of step c) is followed by sterile filtration.
13. Exendin-4 as set forth in SEQ ID No. 1, wherein the Exendin-4 is formulated into dosage form in formulation buffer.

14. The Exendin-4 as claimed in claim 13, wherein the Exendin-4 is obtained by the process as claimed in claim 1; the Exendin-4 has purity of at least 97%, preferably about 99%; and the formulation buffer comprises component selected from group of sodium acetate, acetic acid, D-Mannitol and Meta-cresol or any combinations thereof.