

SOLE INVENTOR



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APPLICATION FOR UNITED STATES LETTERS PATENT

SPECIFICATION

TO ALL WHOM IT MAY CONCERN:

Be it known that I, FU-KUEN LIN

a citizen of the United States, residing at 418 Thunderhead Street, Thousand Oaks,

In the County of Ventura and State of California

have invented a new and useful "PRODUCTION OF ERYTHROPOIETIN"

of which the following is a specification.

WHAT IS CLAIMED IS:

1. A purified and isolated polypeptide having part or all of the primary structural conformation and one or more of the biological properties of naturally-occurring erythropoietin and characterized by being the product of prokaryotic or eukaryotic expression of an exogenous DNA sequence.
2. A polypeptide according to claim 1 further characterized by being free of association with any mammalian protein.
3. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a cDNA sequence.
4. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a manufactured DNA sequence.
5. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a genomic DNA sequence.
6. A polypeptide according to claim 1 wherein the exogenous DNA sequence is carried on an autonomously replicating circular DNA plasmid or viral vector.
7. A polypeptide according to claim 1 possessing part or all of the primary structural conformation of human erythropoietin as set forth in Table VI or any naturally occurring allelic variant thereof.
8. A polypeptide according to claim 1 possessing part or all of the primary structural conformation of monkey erythropoietin as set forth in Table V or any naturally occurring allelic variant thereof.

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35. A DNA sequence coding for [Phe¹⁵]hEPO, [Phe⁴⁹]hEPO, [Phe¹⁴⁵]hEPO, [His⁷]hEPO, [Asp² des-Pro² through Ile⁶]hEPO, [des-Thr¹⁶³ through Arg¹⁶⁶]hEPO, or [Δ27-55]hEPO.

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36. A DNA sequence according to claim 34 which is a manufactured sequence.

37. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to either of claims 14, 17, 34 or 35.

38. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 37.

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Sub 37

39. A polypeptide product of the expression in a procaryotic or eucaryotic host cell of a DNA sequence according to claims 17 or 34.

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40. A glycoprotein product having a primary structural conformation sufficiently duplicative of that of a naturally-occurring erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate composition which differs from that of naturally-occurring erythropoietin.

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Sub 37

41. A glycoprotein product having a primary structural conformation sufficiently duplicative of that of a naturally-occurring human erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate composition which differs from that of naturally-occurring human erythropoietin.

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42. Vertebrate cells which can be propagated in vitro continuously and which upon growth in culture are capable of producing in the medium of their growth in excess of 100 U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay.

43. Vertebrate cells according to claim 42 capable of producing in excess of 500 U erythropoietin per 10^6 cells in 48 hours.

44. Vertebrate cells according to claim 42 capable of producing in excess of 1,000 U erythropoietin per 10^6 cells in 48 hours.

45. Vertebrate cells according to claim 42 which are mammalian or avian cells.

46. Vertebrate cells according to claim 45 which are COS-1 cells or CHO cells.

47. A synthetic polypeptide having part or all of the amino acid sequence as set forth in ~~Table V~~ ^{Figure 5} and having one or more of the in vivo or in vitro biological activities of naturally-occurring monkey erythropoietin.

48. A synthetic polypeptide having part or all of the amino acid sequence set forth in ~~Table VI~~ ^{Figure 6}, other than a sequence of residues entirely within the sequence numbered 1 through 20, and having a biological property of naturally-occurring human erythropoietin.

49. A synthetic polypeptide having part or all of the secondary conformation of part or all of the amino acid sequence set forth in ~~Table VI~~ ^{Figure 6}, other than a sequence of residues entirely within the sequence numbered 1 through 20, and having a biological property of naturally-occurring human erythropoietin.