ENGINEERED BACTERIUM FOR PRODUCING KGF-2 PROTEIN, PREPARATION METHOD THEREFOR, AND USE THEREOF

FIELD OF TECHNOLOGY

[0001] The present invention relates to the technical field of genetic engineering, and specifically relates to an engineered bacterium for producing a KGF-2 protein, a preparation method therefor, and use thereof.

BACKGROUND

[0002] Keratinocyte growth factor-2 (KGF-2), also known as fibroblast growth factor-10 (FGF-10), is the 10th member of the Fibroblast Growth Factor (FGFs) family and is an endogenous polypeptide growth factor present in the body. KGF-2 is a single-copy gene consisting of 3 exons and 2 introns, located on chromosome 15. KGF-2 is a single polypeptide chain containing 5 cysteine residues, of which 4 cysteines form two pairs of disulfide bonds, while the remaining cysteine is in a folded cloverleaf-shaped peptide. The mature human KGF-2 protein consists of 171 amino acids, with a theoretical molecular weight of 19.4 kD. The human KGF-2 gene sequence published in GenBank has the accession number NM_004465.1.

[0003] KGF-2 exhibits high specificity for epithelial tissues and possesses physical, chemical, and biological stability. KGF-2 can not only promote the healing of corneal epithelial injuries, but also does not stimulate the growth of corneal neovascularization, thus making it suitable for the treatment of corneal epithelial injuries. KGF-2 promotes the growth of epidermal keratinocytes, repairs damaged cells, and accelerates cellular metabolism. Through the specific repair of keratinocytes by KGF-2, the skin's immunity and resistance are enhanced, restoring the skin to a healthy and bright-white state. Moreover, KGF-2 is also widely used in the preparation of cosmetics or pharmaceuticals capable of repairing tissues.

[0004] Currently, the recombinant human keratinocyte growth factor-2 (rhKGF-2) is generally obtained from KGF-2 by genetic engineering methods, and is typically expressed for production using an *Escherichia coli* expression system. Moreover, rhKGF-2 can be expressed and produced through various methods, including inclusion body expression,

soluble expression, and secretory expression, etc. In the prior art (studies on the fermentation conditions of rhKGF-2 engineered bacteria), a KGF-2 was constructed. The rhKGF-2 protein is produced by fermenting an *Escherichia coli* engineered bacterium [pET26b/KGF-2/BL(DE)21]. After the scale-up production of a fermenter, the yield of the rhKGF-2 protein is only about 30%. Moreover, ampicillin is added to the fermentation medium, indicating that the *Escherichia coli* engineered bacterium used for producing the rhKGF-2 protein is ampicillin-resistant.

[0005] However, the use of engineered bacteria carrying ampicillin for producing KGF-2 no longer complies with the requirements of the *Chinese Pharmacopoeia*. Furthermore, the engineered bacteria used in the prior art for producing the KGF-2 protein exhibit low protein expression levels, with a yield of only about 30%. Meanwhile, their plasmid stability is poor, with a plasmid loss rate exceeding 50% in the later stage of fermentation, making it impossible to achieve stable subculture production of the KGF-2 protein.

[0006] Therefore, there is an urgent need for an engineered bacterium that does not require ampicillin screening and, more importantly, exhibits high KGF-2 protein expression levels and excellent plasmid stability, thereby enabling efficient production of the KGF-2 protein.

SUMMARY

[0007] The purpose of the present invention is to overcome the above deficiencies of the prior art and provide an engineered bacterium for producing a KGF-2 protein, and a preparation method therefor, and use thereof.

[0008] The first objective of the present invention is to provide an engineered bacterium for producing a KGF-2 protein.

[0009] The second objective of the present invention is to provide use of the aforesaid engineered bacterium in producing a KGF-2 protein.

[0010] The third objective of the present invention is to provide a method for producing a KGF-2 protein.

[0011] To achieve the above objectives, the present invention is achieved through the

following solutions:

[0012] An engineered bacterium for producing a KGF-2 protein, where the engineered bacterium is *Escherichia coli* transfected with a recombinant vector.

[0013] The recombinant vector is a pET-30a vector carrying a KGF-2 protein coding sequence, a pET-28a vector carrying a KGF-2 protein coding sequence, or a pET-42a vector carrying a KGF-2 protein coding sequence. The KGF-2 protein has an amino acid sequence of SEQ ID NO: 2.

[0014] The *Escherichia coli* is OverExpress C43(DE3) *Escherichia coli*, Rosetta(DE3) *Escherichia coli*, or BL21 Star(DE3) *Escherichia coli*.

[0015] Preferably, the recombinant vector is a pET-30a vector carrying the KGF-2 protein coding sequence, and the *Escherichia coli* is Rosetta (DE3) *Escherichia coli* or OverExpress C43(DE3) *Escherichia coli*.

[0016] More preferably, the KGF-2 protein coding sequence has a nucleotide sequence of SEQ ID NO: 1.

[0017] More preferably, the recombinant vector is a pET-30a vector carrying the KGF-2 protein coding sequence, and the *Escherichia coli* is Rosetta (DE3) *Escherichia coli*.

[0018] The present invention further sets forth use of the aforesaid engineered bacterium in producing a KGF-2 protein.

[0019] The aforesaid engineered bacterium not only does not require ampicillin screening, but also exhibits high KGF-2 protein expression levels. Meanwhile, plasmid stability is maintained during the fermentation process for KGF-2 protein production, thus enabling stable expression of KGF-2 protein. The yield of the KGF-2 protein produced using the above engineered bacterium is as high as 40%-50%.

[0020] The present invention further sets forth a method of producing a KGF-2 protein. The method utilizes any of the aforesaid engineered bacteria for fermentation production of the KGF-2 protein.

[0021] Preferably, the method includes the following steps:

[0022] step S1, Any of the engineered bacteria is inoculated into an LB medium in a volume ratio of 1:20-200, and adequately cultured to obtain a primary seed solution. The primary seed solution is inoculated into the LB medium in a volume ratio of 1:20-200, and

cultured adequately to obtain a secondary seed culture solution.

[0023] step S2. The secondary seed culture solution obtained in the step S1 is mixed with a basal fermentation medium in a volume ratio of 2-10:20-100 to obtain a culture solution. The pH value of the culture solution is adjusted to 6.2-8.2, and culturing is performed under conditions of a dissolved oxygen of greater than 10%, a temperature of 25-45°C, a rotating speed of 100-1,600 rpm, and an aeration rate of 5-100 L/min. When an OD_{600} of the culture solution reaches 10-50, IPTG at a final concentration of 0.1-3.0 mM and a feed solution accounting for 1.0-30% of a total volume are added to the culture solution, and adequately cultured to obtain fermented engineered bacterial cells.

[0024] The basal fermentation medium is obtained by mixing a basal medium 1, a basal medium 2, and 1-20 g/L of a thiamine solution in a ratio of 15-25 L:0.5-1.5 L:6-10 mL.

[0025] The basal medium 1 includes: at final concentrations, 15-25 g/L peptone, 15-25 g/L yeast powder, 3-7 g/L sodium chloride, 1-3 g/L potassium dihydrogen phosphate, 2-4 g/L dipotassium hydrogen phosphate, and 1-3 g/L ammonium chloride. The basal medium 2 includes, at final concentrations, 2-10 g/L glucose monohydrate, 0.2-0.8 g/L anhydrous magnesium sulfate, and 0.01-0.09 g/L calcium chloride.

[0026] The feed solution includes: at final concentrations, 15-25 g/L peptone, 15-25 g/L yeast powder, 3-7 g/L sodium chloride, 1-3 g/L potassium dihydrogen phosphate, and 2-4 g/L dipotassium hydrogen phosphate trihydrate.

[0027] step S3. The fermented engineered bacterial cells obtained in the step S2 are homogenized and disrupted, and subjected to solid-liquid separation to collect a supernatant for CM chromatography to obtain a crudely purified KGF-2 protein. Heparin chromatography is performed on the crudely purified KGF-2 protein, to obtain the KGF-2 protein.

[0028] Preferably, the LB medium in the step S1 is an LB medium containing, at final concentrations, 2-10 g/L yeast extract powder, 9-21 g/L peptone, and 9-21 g/L sodium chloride, with a pH value of 6.2-8.2.

[0029] More preferably, the LB medium in the step S1 is an LB medium containing the yeast extract powder at a final concentration of 5.0 g/L, peptone at 10.0 g/L, sodium chloride at 10.0 g/L, with a pH value of 6.2-8.2.

[0030] Preferably, the thiamine solution in the step S2 is an aqueous thiamine

solution.

[0031] Preferably, in the step S2: when the OD_{600} of the culture solution reaches 40, IPTG at a final concentration of 0.1-3.0 mM and a feed solution at 1.0-30% of the total volume are added.

[0032] Preferably, the homogenizing and disrupting is specifically as follows: fully mixing the fermented engineered bacterial cells obtained in the step S2 with Buffer A in a ratio of 1 g:1-100 mL for homogenization and disruption at 4-40°C.

[0033] The Buffer A has a formulation of: 15-45 mM phosphate buffer, 0.2-1.8 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0034] More preferably, the Buffer A has a formulation of: 30 mM phosphate buffer (PB), 0.2 M sodium chloride, and 20 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0035] Preferably, the CM chromatography in the step S3 is specifically as follows:

[0036] The CM chromatographic column is equilibrated, and the supernatant obtained in the step S3 is added to the equilibrated CM chromatographic column at 0.01-10.0 g/mL. The flow-through peak is collected. The CM chromatographic column is re-equilibrated with the Buffer A, and impurities are washed thoroughly with Buffer E, followed by eluting 0.5-10 column volumes with the Buffer C. Eluate is collected when $UV_{280} > 20$ mAU, to obtain the crudely purified KGF-2 protein.

[0037] The Buffer C has a formulation of: 15-45 mM phosphate buffer, 0.2-1.8 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0038] The Buffer C has a formulation of: 15-45 mM phosphate buffer (PB), 0.2-1.8 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0039] More preferably, the equilibrating the CM chromatographic column is specifically as follows: Buffer A is added to the CM chromatographic column to equilibrate 0.5-10 column volumes until the baseline of the CM chromatographic column is stable, and the conductivity is not greater than 100 ms/cm, the pH value of the effluent of the CM chromatographic column is 6.2-8.2, and the UV_{280} absorption baseline is stable, to obtain the equilibrated CM chromatographic column.

[0040] More preferably, 4 column volumes are eluted with Buffer C.

[0041] More preferably, the Buffer C has a formulation of: 30 mM phosphate buffer

(PB), 0.6 M sodium chloride, and 20 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0042] The Buffer E has a formulation of: 30 mM phosphate buffer (PB), 0.3 M sodium chloride, and 20 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0043] Preferably, the Heparin chromatography in the step S3 is specifically as follows:

[0044] The heparin chromatographic column is equilibrated. The crudely purified KGF-2 protein obtained in the step S3 is mixed with Buffer A at equal volumes, and the equilibrated heparin chromatographic column is loaded at 0.10-2.00 g/mL, and then the flow-through peak is collected. Afterwards, the heparin chromatographic column is re-equilibrated with Buffer B. The column is washed thoroughly with Buffer C to remove impurities, and then eluted with Buffer D for 0.5-10 column volumes. When UV_{280} > 20 mAU, the eluate is collected, to obtain the KGF-2 protein.

[0045] The Buffer B has formulation of: 15-45 mM phosphate buffer (PB), 0.1-3 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0046] The Buffer D has a formulation of: 15-45 mM phosphate buffer (PB), 0.1-3 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0047] More preferably, the heparin chromatographic column is specifically equilibrated as follows: adding Buffer B to the heparin chromatographic column to equilibrate 0.5-10 column volumes until the baseline of the heparin chromatographic column is stable and the conductivity is not greater than 100 ms/cm, the pH of the effluent of the heparin chromatographic column is 6.2-8.2, and the UV_{280} absorption baseline is stable, to obtain the equilibrated heparin chromatographic column.

[0048] More preferably, 2 column volumes are eluted with Buffer D.

[0049] More preferably, the Buffer B has a formulation of: 30 mM phosphate buffer (PB), 0.4 M sodium chloride, and 20 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0050] The Buffer D has a formulation of: 30 mM phosphate buffer (PB), 0.8 M sodium chloride, and 20 mM EDTA-2Na, with a pH value of 6.2-8.2.

[0051] Compared with the prior art, the present invention has the following beneficial effects:

[0052] The present invention provides an engineered bacterium for producing a

KGF-2 protein. The engineered bacterium is *Escherichia coli* transfected with a recombinant vector. The recombinant vector is a pET-30a vector carrying a KGF-2 protein coding sequence, pET-28a vector carrying a KGF-2 protein coding sequence, or pET-42a vector carrying the KGF-2 protein coding sequence. The *Escherichia coli* is OverExpress C43(DE3) *Escherichia coli*, Rosetta(DE3) *Escherichia coli*, or BL21 Star(DE3) *Escherichia coli*. When the engineered bacterium is used for producing the KGF-2 protein, the KGF-2 expression level is high. Meanwhile, the plasmid remains stable during the fermentation process of the KGF-2 protein production, thereby achieving stable expression of the KGF-2 protein and achieving the purpose of long-term fermentation production of the KGF-2 protein. Furthermore, the yield of the KGF-2 protein produced by fermentation using the engineered bacterium is as high as 40%-50%, increasing about 66% over the current yield, and thus has an excellent application value.

BRIEF DESCRIPTION OF THE DRAWINGS

[0053] FIG. 1 is a chromatogram of 500109-pET-28a(+) in Example 1;

[0054] FIG. 2 is a chromatogram of 500109-pET-30a(+) in Example 1;

[0055] FIG. 3 is a chromatogram of 500109-pET-42a(+) in Example 1;

[0056] FIG. 4 is an observation image of a cultured 500109-S9 engineered bacterium in Example 1;

[0057] FIG. 5 is an observation result map of the cultured 500109-S9 engineered bacterium under an electron microscope in Example 1;

[0058] FIG. 6 shows an electrophoresis result of recombinant engineered bacteria 500109-S2 to 500109-S5 after being induced for 4 h in Example 1;

[0059] FIG. 7 shows an electrophoresis result of recombinant engineered bacteria 500109-S6 to 500109-S9 after being induced for 4 h in Example 1;

[0060] FIG. 8 shows an electrophoresis result of recombinant engineered bacteria 500109-S10 to 500109-S13 after being induced for 4 h in Example 1;

[0061] FIG. 9 shows an electrophoresis result of recombinant engineered bacteria 500109-S2 to 500109-S5 after being induced for 16 h in Example 1;

[0062] FIG. 10 shows an electrophoresis result of recombinant engineered bacteria

500109-S6 to 500109-S9 after being induced for 16 h in Example 1;

[0063] FIG. 11 shows an electrophoresis result of recombinant engineered bacteria 500109-S10 to 500109-S13 after being induced for 16 h in Example 1;

[0064] FIG. 12 shows an SDS-PAGE electrophoresis result when the induction temperature is changed in Example 1;

[0065] FIG. 13 shows an SDS-PAGE electrophoresis result when the inducer concentration is changed in Example 1;

[0066] FIG. 14 shows growth curves of the recombinant engineered bacterium with different inoculum sizes in Example 3;

[0067] FIG. 15 shows SDS-PAGE results of the recombinant engineered bacterium with different inoculum sizes in Example 3;

[0068] FIG. 16 shows growth curves of the recombinant engineered bacterium with different basal fermentation media in Example 3;

[0069] FIG. 17 shows an SDS-PAGE result of the recombinant engineered bacterium with different basal fermentation media in Example 3;

[0070] FIG. 18 shows growth curves of the recombinant engineered bacterium added with an inducer at different time in Example 3;

[0071] FIG. 19 shows an SDS-PAGE result of the recombinant engineered bacterium added with an inducer at different time in Example 3;

[0072] FIG. 20 shows growth curves of the recombinant engineered bacterium at different inducer concentrations in Example 3;

[0073] FIG. 21 shows an SDS-PAGE result of the recombinant engineered bacterium at different inducer concentrations in Example 3;

[0074] FIG. 22 shows growth curves of the recombinant engineered bacterium at different temperatures in Example 3;

[0075] FIG. 23 shows an SDS-PAGE result of the recombinant engineered bacterium at different temperatures in Example 3;

[0076] FIG. 24 shows a low-column cation exchange chromatogram in Example 3;

[0077] FIG. 25 shows a high-column cation exchange chromatogram in Example 3;

[0078] FIG. 26 shows a low-column affinity chromatogram in Example 3;

[0079] FIG. 27 shows a high-column affinity chromatogram in Example 3;

[0080] FIG. 28 shows an SDS-PAGE result at different column heights in Example 3;

[0081] FIG. 29 shows a cation exchange chromatogram at a purification temperature of 10°C in Example 3;

[0082] FIG. 30 shows an affinity chromatogram at a purification temperature of 10°C in Example 3;

[0083] FIG. 31 shows an SDS-PAGE result at a purification temperature of 10°C in Example 3;

[0084] FIG. 32 shows a cation exchange chromatogram after impurity washing off in Example 3;

[0085] FIG. 33 shows an affinity chromatogram after impurity washing in Example 3;

[0086] FIG. 34 shows an SDS-PAGE result after impurity washing in Example 3;

[0087] FIG. 35 shows a cation exchange chromatogram after impurity washing with urea in Example 3;

[0088] FIG. 36 shows an affinity chromatogram after impurity washing with urea in Example 3;

[0089] FIG. 37 shows an SDS-PAGE result of Control Group 3 in Example 3;

[0090] FIG. 38 shows a cation exchange chromatogram at pH 7.2 in Example 3;

[0091] FIG. 39 shows an affinity chromatogram at pH 6.8 in Example 3;

[0092] FIG. 40 shows an affinity chromatogram at pH 7.0 in Example 3;

[0093] FIG. 41 shows an affinity chromatogram at pH 7.4 in Example 3;

[0094] FIG. 42 shows an SDS-PAGE result at pH 6.8 in Example 3;

[0095] FIG. 43 shows an SDS-PAGE result at pH 7.0 in Example 3;

[0096] FIG. 44 shows an SDS-PAGE result at pH 7.4 in Example 3;

[0097] FIG. 45 shows an affinity chromatogram of Control Group 7 in Example 3;

[0098] FIG. 46 shows an SDS-PAGE result of the finely purified rhKGF-2 protein from Control Group 7 in Example 3;

[0099] FIG. 47 shows a cation exchange chromatogram of Control Group 8 in Example 3;

[0100] FIG. 48 shows an affinity chromatogram of Control Group 8 in Example 3;

[0101] FIG. 49 shows an SDS-PAGE result of Control Group 8 in Example 3;

[0102] FIG. 50 shows a cation exchange chromatogram of Experimental Group 5 in Example 3;

[0103] FIG. 51 shows an affinity chromatogram of Experimental Group 5 in Example 3;

[0104] FIG. 52 shows an affinity chromatogram of Control Group 9 in Example 3;

[0105] FIG. 53 shows an affinity chromatogram of Control Group 10 in Example 3;

[0106] FIG. 54 shows an SDS-PAGE result with different sample loading capacity in Example 3;

[0107] FIG. 55 shows a cation exchange chromatogram of Control Group 11 in Example 3;

[0108] FIG. 56 shows an affinity chromatogram of Control Group 11 in Example 3;

[0109] FIG. 57 shows an SDS-PAGE result with different elution buffers in Example 3;

[0110] FIG. 58 shows a cation exchange chromatogram of Control Group 12 in Example 3;

[0111] FIG. 59 shows an affinity chromatogram of Control Group 12 in Example 3;

[0112] FIG. 60 shows an SDS-PAGE result with buffers of different pH values in Example 3;

[0113] FIG. 61 shows a cation exchange chromatogram of Experimental Group 7 in Example 3;

[0114] FIG. 62 shows an affinity chromatogram of Experimental Group 7 in Example 3;

[0115] FIG. 63 shows an SDS-PAGE result after adding membrane exchange after chromatography in Example 3;

[0116] FIG. 64 shows a cation exchange chromatogram of Experimental Group 8 in Example 3;

[0117] FIG. 65 shows an affinity chromatogram of Experimental Group 8 in Example 3;

[0118] FIG. 66 shows an SDS-PAGE result at different rhKGF-2 protein

concentrations in Example 3;

[0119] FIG. 67 shows a cation exchange chromatogram of Experimental Group 9 in Example 3;

[0120] FIG. 68 shows an affinity chromatogram of Experimental Group 9 in Example 3;

[0121] FIG. 69 shows a SP chromatogram of Experimental Group 9 in Example 3;

[0122] FIG. 70 shows an SDS-PAGE result of a heparin chromatographic eluate and a retentate from Experimental Group 9 in Example 3;

[0123] FIG. 71 shows an SDS-PAGE result of a SP eluate from Experimental Group 9 in Example 3;

[0124] FIG. 72 shows an SDS-PAGE result in Example 4; and

[0125] FIG. 73 shows an SDS-PAGE result of the finely purified rhKGF-2 protein from Comparative Example 1.

DESCRIPTION OF THE EMBODIMENTS

[0126] The present invention will be further specifically set forth below in conjunction with the accompanying drawings and detailed embodiments of the description. The embodiments are merely intended to interpret the present invention, but are not construed as limiting the scope of the present invention. Unless otherwise specified, the experimental methods used in the following embodiments are all conventional methods. The materials and reagents used, unless otherwise specified, are commercially available reagents and materials.

[0127] **Example 1 Screening and construction of the engineered bacterium for producing the KGF-2 protein**

[0128] The human KGF-2 gene sequence (NM_004465.2) published in NCBI serves as the reference genome, to synthesize a nucleotide sequence of the 500109 gene (KGF-2 protein coding sequence) as shown in SEQ ID NO: 1. The amino acid sequence of the encoded KGF-2 protein is as shown in SEQ ID NO: 2.

[0129] Step I. Construction of the expression vector

[0130] The 500109 gene having the nucleotide sequence as shown in SEQ ID NO: 1 was taken as a template; an Xba1I restriction site was introduced at the 5' end and an XhoI

restriction site was introduced at the 3' end, to obtain an inserted gene 1 with a nucleotide sequence as shown in SEQ ID NO: 3. The 500109 gene having the nucleotide sequence as shown in SEQ ID NO: 1 was taken as a template; an NdeI restriction site was introduced at the 5' end and an XhoI restriction site was introduced at the 3' end, to obtain an inserted gene 2 with a nucleotide sequence as shown in SEQ ID NO: 4.

[0131] The inserted gene 1 (SEQ ID NO: 3) and the pET-28a(+) vector were digested with Xba1I and XhoI restriction enzymes, respectively, and then ligated using a T4 ligase to obtain the pET-28a vector carrying the gene with the nucleotide sequence as shown in SEQ ID NO: 1, i.e., 500109-pET-28a(+), with the chromatogram as shown in FIG. 1.

[0132] The inserted gene 2 (SEQ ID NO: 4) and the pET-30a(+) vector were digested with NdeI and XhoI restriction enzymes, respectively, and then ligated using a T4 ligase to obtain the pET-30a(+) vector carrying the gene with the nucleotide sequence as shown in SEQ ID NO: 1, i.e., 500109-pET-30a(+), with the chromatogram as shown in FIG. 2.

[0133] The pET-30a(+) vector was replaced with the pET-42a(+) vector and treated in the same manner to obtain the pET-42a(+) vector carrying the gene with the nucleotide sequence as shown in SEQ ID NO: 1, i.e., 500109-pET-42a(+), with the chromatogram as shown in FIG. 3.

[0134] Step II. Construction of the engineered bacterium

[0135] 1. Method

[0136] BL21(DE3)plysS competent cells (Weidi Biotech EC1003), BL21 Star(DE3) competent cells (Thermo 44-00049), Rosetta(DE3) competent cells (Solarbio C1420), and OverExpress C43(DE3) competent cells (Weidi Biotech EC1040) were used as host bacteria, respectively.

[0137] Taking BL21(DE3)plysS competent cells as the host bacteria as an example: The host bacteria were placed in a centrifugal tube and treated in an ice bath for 15 min. The 500109-pET-28a(+) obtained in the Step I was then mixed with the host bacteria in a volume ratio of 100:6 well, treated in the ice bath for 20 min, and after being treated on the ice bath, treated on a 42°C water bath for 90 s, then put back into the ice bath for 2 min. After the centrifugal tube was treated in the ice bath, an antibiotic-free LB liquid medium (containing 5 g/L yeast extract powder, 10 g/L peptone, and 10 g/L sodium chloride) was added to the

centrifugal tube, mixed well, and then the centrifugal tube was placed into a 37°C constant-temperature shaking incubator for culture for 50 min. The bacterial solution was collected and spread onto an LB solid medium plate containing 30 mg/L kanamycin sulfate resistance (containing 5 g/L yeast extract powder, 10 g/L peptone, and 10 g/L sodium chloride). The LB solid medium plate was sealed with a sealing film, and inverted into a 37°C constant temperature incubator for culture for 16 h.

[0138] At the end of the culture, 2 monoclonal strains of uniform size were picked and inoculated into 2 LB liquid media containing 30 mg/L kanamycin sulfate resistance, and cultured in a constant-temperature shaking incubator at 37°C and 220 rpm until OD_{600} was up to 2-3, to obtain the cultured bacterial solution. Glycerol was added to the cultured bacterial solution to a final glycerol concentration of 20% (v/v), and mixed well to obtain a glycerol bacterium of the recombinant engineered bacterium with vector 500109-pET-28a(+) and host bacteria BL21(DE3)plysS competent cells.

[0139] The host bacteria were replaced with BL21 Star(DE3) competent cells, Rosetta(DE3) competent cells, and OverExpress C43(DE3) competent cells in sequence, and treated in the same manner to obtain recombinant engineered bacteria with different competent cells as host bacteria.

[0140] 500109-pET-28a(+) was replaced sequentially with 500109-pET-30a(+) and 500109-pET-42a(+) obtained in the Step I, and treated in the same manner to obtain the corresponding recombinant engineered bacteria. The specific information of different recombinant engineered bacteria is shown in Table 1, with a total of 12 recombinant engineered bacteria prepared.

Table 1 Information of the recombinant engineered bacteria

Serial number	Engineered bacterium No.	Vector	Host bacterium	Preparation quantity
1	500109-S2	500109-pET-28a(+)	BL21(DE3)plysS	20 vials/clone × 2 clones
2	500109-S3	500109-pET-28a(+)	BL21 Star(DE3)	20 vials/clone × 2 clones

3	500109-S4	500109-pET-28a(+)	Rosetta(DE3)	20 vials/clone × 2 clones
4	500109-S5	500109-pET-28a(+)	Over Express C43(DE3)	20 vials/clone × 2 clones
5	500109-S6	500109-pET-30a(+)	BL21(DE3)plysS	20 vials/clone × 2 clones
6	500109-S7	500109-pET-30a(+)	BL21 Star(DE3)	20 vials/clone × 2 clones
7	500109-S8	500109-pET-42a(+)	Over Express C43(DE3)	20 vials/clone × 2 clones
8	500109-S9	500109-pET-30a(+)	Rosetta(DE3)	20 vials/clone × 2 clones
9	500109-S10	500109-pET-42a(+)	BL21(DE3)plysS	20 vials/clone × 2 clones
10	500109-S11	500109-pET-42a(+)	BL21 Star(DE3)	20 vials/clone × 2 clones
11	500109-S12	500109-pET-42a(+)	Rosetta(DE3)	20 vials/clone × 2 clones
12	500109-S13	500109-pET-30a(+)	Over Express C43(DE3)	20 vials/clone × 2 clones

[0141] The 12 recombinant engineered bacteria as shown in Table 1 were subjected to sequencing. The sequencing results show that all the recombinant engineered bacteria carry the gene with the nucleotide sequence as shown in SEQ ID NO: 1.

[0142] The constructed 500109-S9 engineered bacterium was set as an example. The constructed 500109-S9 engineered bacterium was spread onto an LB agar plate containing 30 mg/L kanamycin sulfate (containing 5 g/L yeast extract powder, 10 g/L peptone, and 10 g/L sodium chloride), and cultured at 37°C for 16 h. At the end of the culture, photographing was performed for observation. Moreover, the cultured bacterium was stained with crystal violet and observed for morphological characteristics under an electron microscope.

[0143] 2. Experimental results

[0144] The observation image of the cultured 500109-S9 engineered bacterium is shown in FIG. 4, and the observation result of the electron microscope is shown in FIG. 5. The results show that after being cultured, the 500109-S9 engineered bacteria exhibit typical *Escherichia coli* colonial morphology, and no other contaminating bacteria are observed. Moreover, no impurity contamination is observed under the electron microscope.

[0145] Step III. Screening of the engineered bacterium

[0146] 1. Protein expression test on the recombinant engineered bacterium after being induced for 4 h

[0147] (1) Experimental method

[0148] 12 recombinant engineered bacteria as shown in Table 1 of the step II were respectively utilized for fermentation to express the KGF-2 protein. One clone parallel was set for each of the recombinant engineered bacteria (two groups in total). Taking 500109-S2 as an example, the specific steps are as follows:

[0149] Glycerol was added to 500109-S2 to a final glycerol concentration of 20% (v/v), and mixed well to obtain an RCB glycerol bacterium. The RCB glycerol bacterium was inoculated into an LB liquid medium containing 30 mg/L kanamycin sulfate in an inoculum size of 0.5% (v/v), and incubated in a constant-temperature shaking incubator at 37°C and 150 rpm for 16 h, to obtain a primary bacterial solution.

[0150] The primary bacterial solution was inoculated into an LB liquid medium containing 30 mg/L kanamycin sulfate at 1% (v/v), and cultured in a constant-temperature shaking incubator at 37°C and 200 rpm until OD_{600} reached 0.8-1.2 (i.e., the strain was in the logarithmic growth phase). IPTG was added to the medium to a final concentration of 1.5 mM. The medium was then continuously subjected to induction culture at 40°C and 200 rpm for 4 h. At the end of the culture, the bacterial cells were collected after thorough centrifugation, and the protein expression level was detected by SDS-PAGE electrophoresis.

[0151] The clone strain of 500109-S2 was treated in the same manner, and 500109-S3 to 500109-S13 as shown in Table 1 were treated in the same manner. The protein expression level was detected by SDS-PAGE electrophoresis.

[0152] (2) Experimental results

[0153] The SDS-PAGE electrophoresis results of the recombinant engineered bacteria after being induced for 4 h are shown in FIGS. 6-8. FIG. 6 shows the SDS electrophoresis results for 500109-S2 to 500109-S5; lanes 1 and 2 represent the electrophoresis results for 500109-S2; lanes 3 and 4 represent the electrophoresis results for 500109-S3; lanes 5 and 6 represent the electrophoresis results for 500109-S4; lanes 7 and 8 represent the electrophoresis results for 500109-S5, and lane 9 represents the electrophoresis result for the marker. FIG. 7 shows the SDS electrophoresis results for 500109-S6 to 500109-S9; lanes 10 and 11 represent the electrophoresis results for 500109-S6; lanes 12 and 13 represent the electrophoresis results for 500109-S7; lanes 14 and 15 represent the electrophoresis results for 500109-S8; lane 16 represents the electrophoresis result for the marker; and lanes 17 and 18 represent the electrophoresis results for 500109-S9. FIG. 8 shows the SDS electrophoresis results for 500109-S10 to 500109-S13; lanes 19 and 20 represent the electrophoresis results for 500109-S10; lane 21 represents the electrophoresis result for the marker; lanes 22 and 23 represent the electrophoresis results for 500109-S11; lanes 24 and 25 represent the electrophoresis results for 500109-S12; and lanes 26 and 27 represent the electrophoresis results for 500109-S13.

[0154] The size of the KGF-2 protein is approximately 20 kDa. The results show that after being induced for 4 h, all the 12 recombinant engineered bacteria may express the KGF-2 protein. However, the KGF-2 protein expression levels in the three recombinant engineered bacteria (500109-S2, 500109-S6, and 500109-S10) using BL21(DE3)plysS competent cells as the host bacteria are lower as compared to other recombinant engineered bacteria, making them unsuitable as production strains for KGF-2 protein expression. In contrast, the KGF-2 protein expression levels in 500109-S3, 500109-S9, 500109-S11, 500109-S12, and 500109-S13 are relatively higher. Moreover, engineered bacteria 500109-S9 and 500109-S13 have higher expression levels of the KGF-2 protein (the protein bands are more obvious in the electrophoresis results).

[0155] 2. Protein expression test on the recombinant engineered bacterium after being induced for 16 h

[0156] (1) Experimental method

[0157] 12 recombinant engineered bacteria listed in Table 1 of the step II were

utilized for fermentation to express the KGF-2 protein. The difference from the experimental method in Step III. 1.(1) is as follows: IPTG was added to a final concentration of 1.5 mM, and induction culture was continued at 40°C and 200 rpm for 16 h. At the end of the culture, bacterial cells were collected by thorough centrifugation and the protein expression level was detected by SDS-PAGE electrophoresis. The remaining experimental steps were the same.

[0158] (2) Experimental results

[0159] The SDS-PAGE electrophoresis results of the recombinant engineered bacteria after being induced for 16 h are shown in FIGS. 9-11. FIG. 9 shows the SDS electrophoresis results for 500109-S2 to 500109-S5; lanes 1 and 2 represent the electrophoresis results for 500109-S2; lanes 3 and 4 represent the electrophoresis results for 500109-S3; lanes 5 and 6 represent the electrophoresis results for 500109-S4; lanes 7 and 8 represent the electrophoresis results for 500109-S5, and lane 9 represents the electrophoresis result for the marker. FIG. 10 shows the SDS electrophoresis results for 500109-S6 to 500109-S9; lanes 10 and 11 represent the electrophoresis results for 500109-S6; lanes 12 and 13 represent the electrophoresis results for 500109-S7; lanes 14 and 15 represent the electrophoresis results for 500109-S8; lane 16 represents the electrophoresis result for the marker; and lanes 17 and 18 represent the electrophoresis results for 500109-S9. FIG. 11 shows the SDS electrophoresis results for 500109-S10 to 500109-S13; lanes 19 and 20 represent the electrophoresis results for 500109-S10, lane 21 represents the electrophoresis result for the marker; lanes 22 and 23 represent the electrophoresis results for 500109-S11; lanes 24 and 25 represent the electrophoresis results for 500109-S12; and lanes 26 and 27 represent the electrophoresis results for 500109-S13. The highlighted bands in the electrophoresis image of FIG. 10 are caused by gel rupture during destaining, not affecting the interpretation of the electrophoresis results. The electrophoresis results shown in FIGS. 9-11 are only the expression situation of the protein of interest via visual observation and thus, are not used for quantitative analysis.

[0160] The results show: After being induced for 16 h, all the 12 recombinant engineered bacteria may express the KGF-2 protein. However, the KGF-2 protein expression levels in 500109-S11 and 500109-S12 are not high, while the KGF-2 protein expression levels in 500109-S3, 500109-S9, and 500109-S13 are significantly higher than in other

recombinant engineered bacteria.

[0161] 3. Detection on the plasmid loss rate in the recombinant engineered bacteria

[0162] (1) Experimental method

[0163] 500109-S3, 500109-S9, 500109-S13, and 500109-S6 were taken as test strains. A total of 8 bacteria solutions of the 4 recombinant engineered bacteria were collected respectively, after 8 h of induction (the induction culture time was changed to 8 h according to the method described in step III.1) and after 16 h of induction (induced according to the method described in step III.2). Each bacteria solution was diluted to 10^6 bacteria/mL and spread onto an antibiotics-free LB agar medium (containing 5 g/L yeast extract powder, 10 g/L peptone, and 10 g/L sodium chloride). After incubation at 37°C for 16 h, 100 single colonies were picked from each medium and inoculated onto an LB agar medium containing 30 mg/L kanamycin sulfate and an antibiotics-free LB agar medium, with 50 single colonies inoculated onto each medium. After incubation at 37°C for 16 h, the number of the colonies on each medium was counted to obtain the number of colonies grown on the resistant medium and the number of colonies grown on the non-resistant medium.

[0164] The plasmid loss rate was calculated based on the number of colonies grown on the resistant and non-resistant media, in combination with the plasmid loss rate inspection method (based on Appendix IX G, Volume III, 2015 edition of the *Chinese Pharmacopoeia*).

[0165] (2) Experimental results

[0166] The detection results of the plasmid loss rate are shown in Table 2.

Table 2 Detection results of the plasmid loss rate

Engineered bacterium No.	Plasmid loss rate in the bacteria induced for 8 h	Plasmid loss rate in the bacteria induced for 16 h
500109-S3	Loss of 2%	Loss of 11%
500109-S6	Loss of 1%	Loss of 1%
500109-S9	Loss of 0%	Loss of 8%
500109-S13	Loss of 0%	Loss of 2%

[0167] The results are as follows: The plasmid stability of the four recombinant engineered bacteria is relatively good, and the plasmid loss rates in the strains induced for 16

h are all less than 50%. Moreover, after being induced for 8 h, both 500109-S9 and 500109-S13 exhibit a plasmid loss rate of 0%, demonstrating superior plasmid stability in 500109-S9 and 500109-S13.

[0168] 4. Optimization of conditions for the recombinant engineered bacteria expressing and producing KGF-2 protein

[0169] (1) Experimental method

[0170] 1) Optimization of the induction temperature, taking 500109-S6 as an example, specifically as follows:

[0171] Glycerol was added to 500109-S6 to a final concentration of 20% (v/v), mixed thoroughly to obtain RCB glycerol bacterium. The RCB glycerol bacterium was inoculated into an LB liquid medium containing 30 mg/L kanamycin sulfate in an inoculum size of 0.5% (v/v), and incubated in a constant-temperature shaking incubator at 30°C and 150 rpm for 16 h, to obtain a primary bacterial solution 1.

[0172] The primary bacterial solution 1 was inoculated into an LB liquid medium containing 30 mg/L kanamycin sulfate at 1% (v/v), and cultured in a constant-temperature shaking incubator at 37°C and 200 rpm until OD600 reached 0.5-1.2 (i.e., the strain was in the logarithmic growth phase). IPTG was added to the medium to a final concentration of 1.5 mM. The medium was then continuously cultured at 40°C and 200 rpm for 12 h. At the end of the culture, the bacterial cells were collected after thorough centrifugation, and the protein expression level was detected by SDS-PAGE electrophoresis.

[0173] The induction temperature after IPTG addition was changed to 45°C, with other steps unchanged. SDS-PAGE electrophoresis was used to detect protein expression levels. 500109-S6 was replaced with 500109-S9 and 500109-S13, respectively, with other steps unchanged. SDS-PAGE electrophoresis was used to detect protein expression levels.

[0174] 2) Optimization of the inducer concentration, specifically as follows:

[0175] Glycerol was added to 500109-S9 to a final glycerol concentration of 20% (v/v), mixed thoroughly to obtain RCB glycerol bacterium 2. The RCB glycerol bacterium was inoculated into an LB liquid medium containing 30 mg/L kanamycin sulfate resistance in an inoculum size of 0.5% (v/v), and incubated in a constant-temperature shaking incubator at 30°C and 150 rpm for 16 h, to obtain a primary bacterial solution 2.

[0176] The primary bacterial solution 2 was inoculated into an LB liquid medium containing 30 mg/L kanamycin sulfate at 1% (v/v), and cultured in a constant-temperature shaking incubator at 37°C and 200 rpm until OD_{600} reached 0.5-2.0 (i.e., the strain was in the logarithmic growth phase). IPTG was added to the medium to a final concentration of 1.5 mM. The medium was then continuously cultured at 40°C and 200 rpm for 10 h. At the end of the culture, the bacterial cells were collected after thorough centrifugation, and the protein expression level was detected by SDS-PAGE electrophoresis.

[0177] The final IPTG concentration was changed to 2.0 mM, with other steps unchanged. SDS-PAGE electrophoresis was used to detect protein expression levels.

[0178] (2) Experimental results

[0179] When the induction temperature is changed, the SDS-PAGE electrophoresis results are shown in FIG. 12. Lane 1 represents the electrophoresis result for the marker; lane 2 represents the electrophoresis result for 500109-S6 induced at 40°C; lane 3 represents the electrophoresis result for 500109-S6 induced at 45°C; lane 4 represents the electrophoresis results for 500109-S9 induced at 40°C; lane 5 represents the electrophoresis result for 500109-S9 induced at 45°C; lane 6 represents the electrophoresis results for 500109-S13 induced at 40°C; and lane 7 represents the electrophoresis result for 500109-S13 induced at 45°C.

[0180] The results are as follows: As for the 500109-S9 and 500109-S13, the KGF-2 protein expression levels are higher at an induction temperature of 40°C as compared to 45°C. Furthermore, when the induction temperature is 40°C, the KGF-2 protein expression level in 500109-S9 is significantly higher than that in other recombinant engineered bacteria.

[0181] When the inducer concentration is changed, the SDS-PAGE electrophoresis results are shown in FIG. 13. Lane 1 represents the electrophoresis result for the marker; lanes 2 and 3 represent the electrophoresis results for 500109-S9 induced at a final IPTG concentration of 1.5 mM; and lanes 4 and 5 represent the electrophoresis results for 500109-S9 induced at a final IPTG concentration of 2.0 mM.

[0182] The results are as follows: Regardless of whether the final concentration of IPTG (inducer) is 1.5 mM or 2.0 mM, there is no significant difference in the KGF-2 protein expression level. Increasing the concentration of IPTG (inducer) may not enhance the KGF-2

protein expression level.

[0183] In summary, the recombinant engineered bacterium 500109-S9, namely the recombinant engineered bacterium with Rosetta(DE3) as the host bacterium and transformed with the 500109-pET-30a(+) vector, not only contains the kanamycin resistance gene but also exhibits higher expression levels of KGF-2 protein as compared to other recombinant engineered bacteria. Meanwhile, the recombinant engineered bacterium further possesses excellent plasmid stability and thus, may be used as an engineered bacterium for producing and expressing the KGF-2 protein. Furthermore, 500109-S9 achieves the highest protein expression level at an induction temperature of 40°C and a final inducer (IPTG) concentration of 1.5mM.

[0184] **Example 2: A method for producing and purifying the rhKGF-2 protein**

[0185] 1. Method for producing the KGF-2 protein

[0186] step S1, preparation of the primary seed solution: 500109-S9 constructed in Example 1 was mixed with glycerol to a final concentration of 20% (v/v) to obtain glycerol bacterium. The pH value of the LB medium (containing 5 g/L yeast extract powder, 10 g/L peptone, and 10 g/L sodium chloride) was adjusted to 7.2 and transferred to a shake flask. The glycerol bacterium and the LB medium were mixed in a volume ratio of 1:50 and cultured in a shaker at 40°C, 150 rpm for 18 h to obtain the primary seed solution.

[0187] step S2, preparation of the secondary seed solution: The primary seed solution obtained in the step S1 was inoculated into an LB medium in a volume ratio of 1:200 and cultured in a shaker at 40°C, 150 rpm for 8 h to obtain the secondary seed solution.

[0188] step S3, fermentation: After a basal medium 1 was subjected to moist heat sterilization in a main fermenter, sterile thiamine aqueous solution (20 g/L) was added; and a basal medium 2 was then added to the main fermenter, and mixed thoroughly to obtain the basal fermentation medium. The volume ratio of the basal medium 1, basal medium 2, and thiamine was 20-40 L:0.5-1.5 L:6-10 mL.

[0189] The secondary seed solution obtained in the step S2 (containing the 500109-S9 strain) was inoculated into the main fermenter at an inoculum size of 8% (v/v), and the initial fermentation broth volume was recorded. The pH value in the main fermenter was controlled at 7.2. Fermentation was performed at a temperature of 40°C, a dissolved oxygen of 10%, an

aeration rate of 50L/min, and a rotating speed of 1000 rpm. When OD_{600} = 40, IPTG at a final concentration of 1.5 mM (based on the initial fermentation broth) and a feed solution accounting for 8% of total volume (containing 20 g/L peptone, 23 g/L yeast powder, 4 g/L sodium chloride, 1 g/L potassium dihydrogen phosphate, and 3 g/L dipotassium hydrogen phosphate trihydrate) were added. Culture was continued for 6 h, and at the end of the culture, the fermented engineered bacteria were obtained.

[0190] The basal medium 1 includes, at final concentrations, 20 g/L peptone, 23 g/L yeast powder, 4 g/L sodium chloride, 1 g/L potassium dihydrogen phosphate, 3 g/L dipotassium hydrogen phosphate, and 2 g/L ammonium chloride. The basal medium 2 includes, at final concentrations, 5 g/L glucose monohydrate, 0.6 g/L anhydrous magnesium sulfate, and 0.013 g/L calcium chloride.

[0191] step S4, purification:

[0192] Buffer A to Buffer E for column chromatography were prepared sequentially, and the temperature of Buffer A to Buffer E was controlled at 15°C.

[0193] The formulation of Buffer A is: 30 mM PB, 0.2 M NaCl, and 20 mM EDTA-2Na, pH=6.2.

[0194] The formulation of Buffer B is: 30 mM PB, 0.4 M NaCl, and 20 mM EDTA-2Na, pH=6.2.

[0195] The formulation of Buffer C is: 30 mM PB, 0.6 M NaCl, and 20 mM EDTA-2Na, pH=6.2.

[0196] The formulation of Buffer D is: 30 mM PB, 0.8 M NaCl, and 20 mM EDTA-2Na, pH=6.2.

[0197] The formulation of Buffer E is: 30 mM PB, 0.3 M NaCl, and 20 mM EDTA-2Na, pH=6.2.

[0198] The fermented engineered bacteria obtained in the step S3 were mixed with Buffer A at a ratio of 1 g:20 mL, stirred thoroughly to obtain a mixed solution. At 15°C, the mixed solution was homogenized and disrupted once at 200 Bar first, and then homogenized and disrupted twice at 700 Bar. After complete disruption (i.e., no intact bacterial cells in the mixed solution, OD_{600} < 30), the homogenized liquid was collected and centrifuged at 7,000 rpm, 10°C for 20 min. The supernatant was collected and filtered, and the filtrate was

collected.

[0199] CM chromatography: The CM chromatographic column (Cytiva CM Sepharose Fast Flow, column height: 20.2 cm, CV: 107 mL) was connected and equilibrated with Buffer A at a linear velocity of 100 cm/h for three column volumes until the baseline of the CM chromatographic column was stable and the conductivity ≤ 100 ms/cm, the effluent pH value was 7.2 and the UV_{280} absorption baseline was stable. The filtrate (CM loading sample) was loaded at 0.5 g/mL, and the flow-through solution was collected. The CM chromatographic column was re-equilibrated with Buffer A until the UV_{280} absorption baseline was stable. Then, the column was washed with Buffer E for two column volumes. Collection started when UV > 20 mAU and stopped when UV < 20 mAU, to obtain an impurity-washed solution. The peak pattern, UV, and conductivity were observed, and the volume of the impurity peak was recorded. After impurity washing, Buffer C was used for elution for two column volumes. Collection of elution began when UV_{280} >100 mAU and stopped when UV_{280} < 100 mAU. The CM chromatographic eluate was collected, which was the crudely purified rhKGF-2 protein. The CM chromatographic column was then regenerated, cleaned, and stored.

[0200] Heparin chromatography: The Heparin chromatographic column (Cytiva Heparin Sepharose Fast Flow, column height: 15 cm, CV: 80 mL) was collected, and equilibrated with Buffer B at a linear velocity of 60 cm/h for 3 column volumes until the baseline of the Heparin chromatographic column was stable and the conductivity was ≤ 100 ms/cm, the effluent of the Heparin chromatographic column had a pH value of 7.2 and a stable UV_{280} absorption baseline. The CM chromatographic eluate was mixed with Buffer A in equal volumes, and loaded at 0.5 g/mL, and then the flow-through peak was collected. Then, the Heparin chromatographic column was re-equilibrated with Buffer B until the UV_{280} absorption baseline was stable. Next, impurities were washed off with Buffer C, and after the UV_{280} absorption baseline was stable via washing, the heparin impurity-washed solution was collected. After washing off impurities, the column was eluted with Buffer D for 2 column volumes. Collection of the eluate started when UV_{280} >100 mAU, and stopped when UV_{280} <100 mAU, to obtain the Heparin chromatographic eluate (heparin eluate), namely, the finely purified rhKGF-2. The mass of the finely purified rhKGF-2 was determined. The Heparin

chromatographic column was regenerated, cleaned, and stored.

[0201] 2. Detection of the rhKGF-2 protein expression level

[0202] The protein content of the finely purified rhKGF-2 obtained in the S4 of step 1 was determined by a Lowry method. The purified rhKGF-2 obtained in the S4 of step 1 was also detected by high-performance liquid chromatography (HPLC) and SDS-PAGE electrophoresis to determine the purity of the finely purified rhKGF-2 obtained in the S4 of step 1.

[0203] Based on the purity of the finely purified rhKGF-2 and the quality of the finely purified rhKGF-2 protein obtained in the step 1, the mass of the rhKGF-2 protein was calculated.

[0204] 3. Experimental results

[0205] The detection results of the expression level of finely purified rhKGF-2 protein obtained in S4 of the step 1 are shown in Table 3.

Table 3 Detection results of the expression level of finely purified rhKGF-2 protein

	Concentration (mg/mL)	HPLC (%)	SDS-PAGE (%)
Finely purified rhKGF-2 protein	6.03	97.0	97.2

[0206] The results are as follows: High-purity KGF-2 protein may be obtained by producing and purifying the KGF-2 protein using the 500109-S8 constructed in Example 1 and in combination with the method described in step 1. Moreover, the purified rhKGF-2 protein has a mass of 7 g, and a yield of 50% (theoretical yield is 14 g).

[0207] **Example 3 Optimization of the method for producing and purifying the rhKGF-2 protein**

[0208] I. Experimental methods

[0209] 1. Effect of the inoculum size of the recombinant engineering bacterium on the rhKGF-2 protein expression level

[0210] According to the KGF-2 protein production method described in step 1 of Example 2, the inoculum size of the secondary seed solution in S3 was changed to 15% (v/v) for fermentation production and purification of the rhKGF-2 protein (denoted as

F2023040218).

[0211] During the fermentation process, the OD_{600} of substances in the fermenter was recorded every 2 h. The fermentation time serves as the x-axis, and OD_{600} as serves as the y-axis to plot a growth curve of the strain in an inoculum size of 15%. Strains were respectively collected at 2 h, 4 h, 6 h, and 8 h of fermentation. The protein expression levels were detected by SDS-PAGE electrophoresis. The plasmid loss rate was detected by a plasmid loss rate inspection method.

[0212] The method described in step 1 of Example 2 was used as a Control Group. The growth curve, protein expression level, and plasmid loss rate of the strain in an 8% inoculum size (denoted as F2023042321) were detected.

[0213] 2. Influence of the basal fermentation medium on the rhKGF-2 protein expression level

[0214] According to the KGF-2 protein production method described in the Step 1 of Example 2, in the step S3, the basal medium 1 was replaced with an M9Y medium and the basal medium 2 was replaced with 4 g/L carbon source medium for fermentation production and purification of the rhKGF-2 protein (designated as F2023042317). The composition of the M9Y medium is: 10 g/L peptone, 10 g/L yeast powder, 12 g/L potassium dihydrogen phosphate, 3 g/L dipotassium hydrogen phosphate, 2 g/L ammonium sulfate, 1 g/L citric acid monohydrate, 0.02 g/L ferrous sulfate heptahydrate, and 3 g/L anhydrous magnesium sulfate.

[0215] During the fermentation process, the OD_{600} of substances in the fermenter was recorded every 2 h. The fermentation time serves as the x-axis, and OD600 as serves as the y-axis to plot a growth curve of the strain. Strains were respectively collected at 2 h, 4 h, 6 h, and 8 h of fermentation. The protein expression levels were detected by SDS-PAGE electrophoresis. The plasmid loss rate was detected by a plasmid loss rate inspection method.

[0216] The method described in Step 1 of Example 2 was used as a Control Group (designated as F2023042321), to detect the strain growth curve, protein expression level, and plasmid loss rate when using the basal fermentation medium described in Step 1 of Example 2 for fermentation.

[0217] 3. Influence of the inducer (IPTG) addition time on the rhKGF-2 protein expression level

[0218] According to the KGF-2 protein production method described in Step 1 of Example 2, the IPTG addition time in S3 was changed to: OD_{600} = 50, for fermentation production and purification of the rhKGF-2 protein (designated as F2023042318).

[0219] During the fermentation process, the OD_{600} of substances in the fermenter was recorded every 2 h. The fermentation time serves as the x-axis, and OD600 as serves as the y-axis to plot a growth curve of the strain. Strains were respectively collected at 2 h, 4 h, 6 h, and 8 h of fermentation. The protein expression levels were detected by SDS-PAGE electrophoresis. The plasmid loss rate was detected by a plasmid loss rate inspection method.

[0220] The method described in Step 1 of Example 2 was used as a Control Group to detect the strain growth curve, protein expression level, and plasmid loss rate when IPTG was added at OD_{600} = 40 during fermentation (designated as F2023042321).

[0221] 4. Influence of the inducer (IPTG) concentration on the rhKGF-2 protein expression level

[0222] According to the KGF-2 protein production method described in Step 1 of Example 2, the final IPTG concentration in S3 was changed to 2.0 mM for fermentation production and purification of the rhKGF-2 protein (designated as F2023043019).

[0223] During the fermentation process, the OD_{600} of substances in the fermenter was recorded every 2 h. The fermentation time serves as the x-axis, and OD600 as serves as the y-axis to plot a growth curve of the strain. Strains were respectively collected at 2 h, 4 h, 6 h, and 8 h of fermentation. The protein expression levels were detected by SDS-PAGE electrophoresis. The plasmid loss rate was detected by a plasmid loss rate inspection method.

[0224] The method described in Step 1 of Example 2 was used as a Control Group (designated as F2023042321) to detect the strain growth curve, protein expression level, and plasmid loss rate when the final IPTG concentration was 1.5 mM.

[0225] 5. Influence of the induction temperature on the rhKGF-2 protein expression level

[0226] According to the KGF-2 protein production method described in Step 1 of Example 2, the temperature of the fermenter in S3 was adjusted to 45°C (i.e., the induction temperature was maintained at 45°C) for fermentation production and purification of the rhKGF-2 protein (designated as F2023042622).

[0227] During the fermentation process, the OD_{600} of the substances in the fermenter was recorded every 2 h. The fermentation time serves as the x-axis, and OD_{600} as serves as the y-axis to plot a growth curve of the strain. Strains were respectively collected at 2 h, 4 h, 6 h, and 8 h of fermentation. The protein expression levels were detected by SDS-PAGE electrophoresis. The plasmid loss rate was detected by a plasmid loss rate inspection method.

[0228] The method described in Step 1 of Example 2 was used as a control group (designated as F2023042321) to detect the strain growth curve, protein expression level, and plasmid loss rate when the induction temperature was 40°C during fermentation.

[0229] 6. Influence of the chromatographic column height during the purification process on the purification effect of the rhKGF-2 protein

[0230] Experimental Group 1: According to the KGF-2 protein production method described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column having a column height of 13.3 cm, and the Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column having a column height of 7.5 cm. The linear velocity both in CM chromatography and Heparin chromatography was adjusted to 150 cm/h, and no impurity-washed solution (Buffer E) was used for impurity washing during CM chromatography. Other procedures were kept the same, resulting in the rhKGF-2 protein finely purified at a low column height. Chromatograms of the CM chromatography process (low-column cation exchange chromatogram) and chromatograms of the Heparin chromatography process (low-column affinity chromatogram) were recorded.

[0231] According to the method described in Step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the rhKGF-2 protein finely purified at a low column height were determined.

[0232] Control Group 1: The CM chromatographic column having a column height of 13.3 cm was replaced with a CM chromatographic column having a column height of 20.2 cm, and the Heparin chromatographic column having a column height of 7.5 cm was replaced with a Heparin chromatographic column having a column height of 15 cm. The linear velocity in CM chromatography and in Heparin chromatography was set to 120 cm/h and 120 cm/h, respectively, to obtain a rhKGF-2 protein finely purified at high column height.

Chromatograms of the CM chromatography process (high-column cation exchange chromatogram) and the Heparin chromatography process (high-column affinity chromatogram) were recorded.

[0233] According to the method described in Step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the rhKGF-2 protein finely purified at high column height, were determined.

[0234] 7. Influence of the temperature during purification on the purification effect of the rhKGF-2 protein

[0235] Control Group 2: According to the KGF-2 protein production method described in Step 1 of Example 2, the temperature of Buffer A to Buffer D used for column chromatography in S4 was controlled to 10°C, and no impurity-washed solution (Buffer E) was used for impurity washing during CM chromatography. Other procedures were kept the same, resulting in the crudely purified rhKGF-2 protein at a purification temperature of 10°C and the finely purified rhKGF-2 protein at a purification temperature of 10°C. The heparin chromatographic eluate was collected in fractions during heparin chromatography to obtain heparin eluate 1 (collected at 80-791-500 mAU), heparin eluate 2 (500-200 mAU), and heparin eluate 3 (200-800 mAU).

[0236] Chromatograms of the CM chromatography process (cation exchange chromatogram at a purification temperature of 10°C) and the Heparin chromatography process (affinity chromatogram at a purification temperature of 10°C) were recorded. According to the method described in Step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the crudely purified rhKGF-2 protein at a purification temperature of 10°C and the finely purified rhKGF-2 protein at a purification temperature of 10°C were determined. SDS-PAGE electrophoresis detection was performed on the heparin eluent 1, heparin eluent 2, heparin eluent 3, and KGF-2 stock solution (the sample containing only KGF-2 protein).

[0237] 8. Influence of the impurity washing step during CM chromatography on the purification effect of the rhKGF-2 protein

[0238] Experimental Group 2: According to the KGF-2 protein production method described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a

CM chromatographic column having a column height of 21 cm (Cytiva CM Sepharose Fast Flow, column height: 21 cm; CV: 422 mL). The Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column having a column height of 31 cm (Cytiva Heparin Sepharose Fast Flow, column height: 31 cm; CV: 165 mL). Other procedures were kept the same, resulting in the impurity-washed, crudely purified rhKGF-2 protein and the impurity-washed, finely purified rhKGF-2 protein.

[0239] Chromatograms of the CM chromatography process (impurity-washed cation exchange chromatogram) and the Heparin chromatography process (impurity-washed affinity chromatogram) were recorded. According to the method described in Step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the impurity-washed, crudely purified rhKGF-2 protein and the impurity-washed, finely purified rhKGF-2 protein were determined.

[0240] The Control Group 3 differs from Experimental Group 2 in the following: Buffer E also contains 2 M urea. All other steps are exactly the same, resulting in the rhKGF-2 protein after impurity washing with urea and crude purification, and the rhKGF-2 protein after impurity washing with urea and fine purification. Moreover, chromatograms of the CM chromatography process (cation exchange chromatograms after impurity removal with urea) and the Heparin chromatography process (affinity chromatograms after impurity removal with urea) were recorded. According to the method described in Step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 protein impurity removal with urea were determined. At the same time, the CM loading sample, flow-through solution, impurity-washed solution, rhKGF-2 protein after impurity washing with urea and crude purification, and the rhKGF-2 protein after impurity washing with urea and fine purification in the CM chromatography process were subjected to SDS-PAGE purity detection. The protein-free blank sample was detected by SDS-PAGE electrophoresis.

[0241] 9. Influence of the pH value during Heparin chromatography on the purification effect of the rhKGF-2 protein

[0242] Experimental Group 3 was set as follows: According to the production method of the KGF-2 protein described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column (Cytiva CM Sepharose Fast Flow,

column height: 19 cm; CV: 100 mL) having a column height of 19 cm, and the Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column (Cytiva Heparin Sepharose Fast Flow, column height: 25 cm; CV: 50 mL) having a column height of 25 cm. All other treatments remained the same, resulting in the crudely purified rhKGF-2 protein at pH 7.2 during the heparin chromatography process and the finely purified rhKGF-2 protein at pH 7.2 during the heparin chromatography process. The chromatograms of the CM chromatography process (cation exchange chromatogram at pH 7.2) and the heparin chromatography process (affinity chromatogram at pH 7.2) were recorded. The protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 protein at pH 7.2 were determined according to the method described in Step 2 of Example 2.

[0243] The Control Group 4 differs from Experimental Group 3 in the following: The pH values of Buffer A to Buffer D were changed to 7.0, while all other treatments remained unchanged, resulting in the crudely purified rhKGF-2 protein at pH 7.0 during the heparin chromatography process and the finely purified rhKGF-2 protein at pH 7.0 during the heparin chromatography process. The chromatograms of the CM chromatography process (the cation exchange chromatogram at pH 7.0) and the heparin chromatography process (the affinity chromatogram at pH 7.0) were recorded. The protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 protein at pH 7.0 during the heparin chromatography process were determined according to the method described in Step 2 of Example 2. Meanwhile, SDS-PAGE purity detection was performed on the heparin impurity-washed solution during the heparin chromatography process and on the finely purified rhKGF-2 protein at pH 7.0 during the heparin chromatography process.

[0244] The Control Group 5 differs from Experimental Group 3 as follows: The pH values of Buffer A to Buffer D were changed to 6.8, while all other treatments remained unchanged, resulting in the crudely purified rhKGF-2 protein at pH 6.8 during the heparin chromatography process and the finely purified rhKGF-2 protein at pH 6.8 during the heparin chromatography process. The chromatogram of the heparin chromatography process (affinity chromatogram at pH 6.8) was recorded. The protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 protein at pH 6.8 during the heparin chromatography process were determined according to the method described in Step 2 of

Example 2. Meanwhile, SDS-PAGE purity detection was performed on the CM chromatography loading sample, flow-through solution, impurity-washed solution, the crudely purified rhKGF-2 protein at pH 6.8 during the heparin chromatography process, the heparin impurity-washed solution during the heparin chromatography process, and the finely purified rhKGF-2 protein at pH 6.8 during the heparin chromatography process, respectively.

[0245] The Control Group 6 differs from Experimental Group 3 in the following: The pH values of Buffer A to Buffer D were changed to 7.4, while all other treatments remained unchanged, resulting in the crudely purified rhKGF-2 protein at pH 7.4 during the heparin chromatography process and the finely purified rhKGF-2 protein at pH 7.4 during the heparin chromatography process. The chromatogram of the heparin chromatography process (affinity chromatogram at pH 7.4) was recorded. The protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 protein at pH 7.4 during the heparin chromatography were determined according to the method described in Step 2 of Example 2. Meanwhile, SDS-PAGE purity detection was performed on the heparin impurity-washed solution during the heparin chromatography process.

[0246] 10. Influence of the Buffer during heparin chromatography on the purification effect of the rhKGF-2 protein

[0247] Experimental Group 5 was set as follows: According to the production method of the KGF-2 protein described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column (Cytiva CM Sepharose Fast Flow, column height: 19 cm; CV: 100 mL) having a column height of 19 cm. The Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column (Cytiva Heparin Sepharose Fast Flow, column height: 25 cm; CV: 50 mL) having a column height of 25 cm. All other treatments remained unchanged, resulting in the finely purified rhKGF-2 protein of Experimental Group 5.

[0248] Control Group 7 differs from Experimental Group 5 in the following: During heparin chromatography, Buffer C was used to equilibrate three column volumes at a linear velocity of 100 cm/h until the Heparin chromatographic column baseline was stable. Then, the CM chromatographic eluate was loaded at 0.01-10.0 g $_{\text{(bacterial sludge)}}$/mL $_{\text{(CM filler)}}$, resulting in the finely purified rhKGF-2 protein of Control Group 7. The chromatogram of the heparin

chromatography process (affinity chromatogram of Control Group 7) was recorded. The protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 protein of Control Group 7 were determined according to the method described in Step 2 of Example 2.

[0249] Control Group 8 differs from Experimental Group 7 in the following: After the CM chromatographic eluate was loaded at 0.01-10.0 g $_{\text{(bacterial sludge)}}$/mL $_{\text{(CM filler)}}$, the heparin flow-through solution was collected, then Buffer C was used to re-equilibrate three column volumes and the re-equilibrated heparin flow-through solution was collected. Next, Buffer F (Buffer C and Buffer D mixed at a volume ratio of 1:3, NaCl concentration of 0.8 M) was used to elute two column volumes, and the eluate was collected when 100 mAU < UV_{280} < 400 mAU, resulting in the finely purified rhKGF-2 protein of Control Group 8. The chromatograms of the CM chromatography process (cation exchange chromatograms of Control Group 8) and the heparin chromatography process (affinity chromatograms of Control Group 8) were recorded. The protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 protein of Control Group 8 were determined according to the method described in Step 2 of Example 2.

[0250] 11. Influence of the sample loading capacity during heparin chromatography on the purification effect of the rhKGF-2 protein

[0251] The sample loading capacity of heparin chromatography in Experimental Group 5 was 1g/mL $_{\text{(heparin filler)}}$, specifically: According to the KGF-2 protein production method described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column having a column height of 19 cm (Cytiva CM Sepharose Fast Flow, column height: 19 cm; CV: 100 mL), and the Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column having a column height of 25 cm (Cytiva Heparin Sepharose Fast Flow, column height: 25 cm; CV: 50 mL). All other treatments remained unchanged, resulting in the crudely purified rhKGF-2 protein of Experimental Group 5 and the finely purified rhKGF-2 protein of Experimental Group 5. The chromatograms during the CM chromatography process (the cation exchange chromatogram in Experimental Group 5) and during the heparin chromatography process (the affinity chromatogram in Experimental Group 5) were recorded.

[0252] Control Group 9 differs from Experimental Group 5 in the following: During

the heparin chromatography process, after mixing the CM eluate with an equal volume of Buffer A, the sample was loaded at 0.6g/mL $_{\text{(heparin filler)}}$, with all other treatments unchanged, to obtain the finely purified rhKGF-2 protein of Control Group 9. The chromatogram during the heparin chromatography process (the affinity chromatogram in Control Group 9) was recorded.

[0253] Control Group 10 differs from Experimental Group 5 in the following: During the heparin chromatography process, after mixing the CM eluate with an equal volume of Buffer A, the sample was loaded at 0.9g/mL $_{\text{(heparin filler)}}$, with all other treatments unchanged, to obtain the finely purified rhKGF-2 protein of Control Group 10. The chromatogram during the heparin chromatography process (the affinity chromatogram in Control Group 10) was recorded.

[0254] According to the method described in Step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the crudely purified rhKGF-2 protein from Experimental Group 5, the finely purified rhKGF-2 protein from Experimental Group 5, the finely purified rhKGF-2 protein from Control Group 9, and the finely purified rhKGF-2 protein from Control Group 10 were measured, respectively.

[0255] 12. Influence of the elution Buffer during heparin chromatography on the purification effect of the rhKGF-2 protein

[0256] The method in Control Group 11 differs from the method described in Example 2 in the following: During heparin chromatography, Buffer G was used to elute 2 column volumes. Collection of the eluate started when $UV_{280} > 100$ mAU, and stopped when $UV_{280} < 100$ mAU, resulting in the finely purified rhKGF-2 protein of Control Group 11. Buffer G differs from Buffer D in that Buffer G does not contain 20 mM EDTA-2Na. The chromatograms during the CM chromatography process (the cation exchange chromatogram in Control Group 11) and the heparin chromatography process (the affinity chromatogram in Control Group 11) were recorded. According to the method described in Step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 protein from Control Group 11 were measured. Meanwhile, SDS-PAGE electrophoresis detection was performed on the CM loading sample, flow-through solution, impurity-washed solution, crudely purified rhKGF-2 protein from Control Group 11, heparin impurity-washed solution,

and finely purified rhKGF-2 protein from Control Group 11 during the CM chromatography process, respectively.

[0257] 13. Influence of the Buffer pH and addition of Tween 80 during heparin chromatography on the purification effect of the rhKGF-2 protein

[0258] Experimental Group 6 was set as follows: According to the production method of the KGF-2 protein described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column (Cytiva CM Sepharose Fast Flow, column height: 19 cm; CV: 100 mL) having a column height of 19 cm. The Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column (Cytiva Heparin Sepharose Fast Flow, column height: 25 cm; CV: 50 mL) having a column height of 25 cm. All other treatments remained unchanged, resulting in the finely purified rhKGF-2 protein of Experimental Group 6.

[0259] Control Group 12 differs from Experimental Group 6 in the following: The pH values of Buffer A to Buffer E were adjusted to 7.2; Tween 80 was respectively added to Buffer A to Buffer E to a final concentration of 0.05% (w/w), with all other treatments unchanged, to obtain the purified rhKGF-2 protein of Control Group 12. The chromatograms during the CM chromatography process (the cation exchange chromatogram in Control Group 12) and the heparin chromatography process (the affinity chromatogram in Control Group 12) were recorded. According to the method described in Step 2 of Example 2, the protein content and purity (HPLC) of the purified rhKGF-2 protein from Control Group 12 were measured. Meanwhile, SDS-PAGE electrophoresis detection was performed on the CM loading sample, flow-through solution, impurity-washed solution, crudely purified rhKGF-2 protein from Control Group 12, heparin impurity-washed solution, and finely purified rhKGF-2 protein from Control Group 12 during the CM chromatography process, respectively.

[0260] 14. Influence of post-chromatography membrane exchange on the purification effect of the rhKGF-2 protein

[0261] Experimental Group 7 was set as follows: According to the production method of KGF-2 protein described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column having a column height of 19 cm (Cytiva

CM Sepharose Fast Flow, column height: 19 cm; CV: 373 mL). The Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column having a column height of 25 cm (Cytiva Heparin Sepharose Fast Flow, column height: 25 cm; CV: 165 mL), with all other treatments unchanged, to obtain the crudely purified rhKGF-2 protein of Experimental Group 7 and the finely purified rhKGF-2 protein of Experimental Group 7. The chromatograms during the CM chromatography process (the cation exchange chromatogram in Experimental Group 7) and during the heparin chromatography process (the affinity chromatogram in Experimental Group 7) were recorded.

[0262] Control Group 13 was set as follows: According to the production method of KGF-2 protein described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column having a column height of 19 cm (Cytiva CM Sepharose Fast Flow, column height: 19 cm; CV: 373 mL), and the Heparin chromatography column in S4 was replaced with a Heparin chromatography column having a column height of 25 cm (Cytiva Heparin Sepharose Fast Flow, column height: 25 cm; CV: 165 mL). CM chromatography and heparin chromatography were performed, and the Heparin chromatographic eluate was collected. Buffer H (pH=7.2, 30 mM PB) was used as the exchange membrane solution, mixed with the Heparin chromatographic eluate in equal volume, and then exchanged three times using a 2KD dialysis membrane to obtain the finely purified rhKGF-2 protein of Control Group 13. Moreover, the dialysate of Control Group 13 was collected.

[0263] Control Group 14 differs from Control Group 13 in the following: The 2KD dialysis membrane was replaced with a 5KD dialysis membrane, to obtain the finely purified rhKGF-2 protein of Control Group 14 and the dialysate of Control Group 14.

[0264] According to the method described in Step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the crudely purified rhKGF-2 protein from Experimental Group 7, the finely purified rhKGF-2 protein from Experimental Group 7, the finely purified rhKGF-2 protein from Control Group 13, the dialysate of Control Group 13, the finely purified rhKGF-2 protein from Control Group 14, and the dialysate of Control Group 14 were measured, respectively.

[0265] 15. Influence of the rhKGF-2 protein concentration on stability

[0266] Experimental Group 8 was set as follows: According to the production method of KGF-2 protein described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column having a column height of 19 cm (Cytiva CM Sepharose Fast Flow, column height: 19 cm; CV: 373 mL); the Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column having a column height of 25 cm (Cytiva Heparin Sepharose Fast Flow, column height: 25 cm; CV: 165 mL). CM chromatography and heparin chromatography were then performed, the Heparin chromatographic eluate was collected. The chromatograms during the CM chromatography process (the cation exchange chromatogram in Experimental Group 8) and the heparin chromatography process (the affinity chromatogram in Experimental Group 8) were recorded.

[0267] Buffer H (pH=7.2, 30 mM PB) was used as the exchange membrane solution. The Heparin chromatographic eluate was mixed with an equal volume of retentate, then exchanged five times using a 2KD dialysis membrane. The exchange solution was collected and diluted with Buffer H to prepare diluted solutions with rhKGF-2 protein concentrations of 3.3 mg/mL, 3.0 mg/mL, 2.0 mg/mL, 1.0 mg/mL, and 0.5 mg/mL, respectively. After standing at 4°C for 1 d, the diluted solutions of different concentrations were detected by high-performance liquid chromatography (HPLC) and SDS-PAGE, respectively.

[0268] 16. Influence of adding SP chromatography after heparin chromatography on the purification effect of the rhKGF-2 protein

[0269] Experimental Group 9 was set as follows: According to the production method of KGF-2 protein described in Step 1 of Example 2, the CM chromatographic column in S4 was replaced with a CM chromatographic column having a column height of 21.5 cm (Cytiva CM Sepharose Fast Flow, column height: 21.5 cm; CV: 422 mL), and the Heparin chromatographic column in S4 was replaced with a Heparin chromatographic column having a column height of 31 cm (Cytiva Heparin Sepharose Fast Flow, column height: 31 cm; CV: 165 mL). CM chromatography and heparin chromatography were then performed to obtain the finely purified rhKGF-2 protein of Experimental Group 9. The chromatograms during the CM chromatography process (the cation exchange chromatogram in Experimental Group 9) and the heparin chromatography process (the affinity chromatogram in Experimental Group 9) were recorded.

[0270] After heparin chromatography, the Heparin chromatographic eluate was collected and mixed with an equal volume of PB (pH=7.2, 20 mM), exchanged five times using a 3KD dialysis membrane, and the retentate was collected. SDS-PAGE gel electrophoresis detection was performed on both the Heparin chromatographic eluate and the dialysate.

[0271] The SP chromatographic column (Borglon Diamond SP Mustang, column height: 25 cm; CV: 50 mL) was connected and equilibrated with 30 mM PB (pH=7.2) at a linear velocity of 150 cm/h for 2 column volumes until the SP column baseline was stable and conductivity was ≤100 ms/cm. The effluent of the CM chromatographic column had a pH value of 7.2, and the UV_{280} absorption baseline was stable. The retentate was loaded at 3 mg/mL, the flow-through solution was collected. The SP column was re-equilibrated with 30 mM PB (pH=7.2) until the UV_{280} absorption baseline was stable.

[0272] Subsequently, the gradient elution of 40 column volumes was performed using a mixture of 30 mM PB (pH=7.2) and Buffer I (20 mM PB + 1 M NaCl, pH 7.2), and the eluate 1 was collected in fractions. Then, the column was eluted with Buffer I for 3 column volumes, and the eluate 2 was collected in fractions (10 mAu < UV_{280} < 10 mAu), to obtain the SP chromatographic sample 1.

[0273] The SP chromatographic sample 1 was mixed with an equal volume of 30 mM PB (pH=7.2), then diluted with 30 mM PB (pH=7.2) to a final NaCl concentration of 0.6 M to obtain the loading sample.

[0274] The SP chromatographic column was equilibrated with 30 mM PB (pH=7.2) at a linear velocity of 150 cm/h for 3 column volumes until the SP column baseline was stable and the conductivity was ≤100 ms/cm. The effluent pH of the CM chromatographic column was 7.2, and the UV_{280} absorption baseline was stable. The loading sample was loaded at 3 mg/mL, and the SP column was re-equilibrated with 30 mM PB (pH=7.2) until the UV_{280} absorption baseline was stable. Buffer I was used to wash off impurities for 3 column volumes, followed by a gradient elution of 15 column volumes using a mixture of 30 mM PB (pH=7.2) and Buffer I. The SP eluate was collected in fractions, resulting in the rhKGF-2 protein after SP chromatography. The collection of the SP eluate in fractions was as follows: The SP eluates at UV_{280} of 5-10 mAu, 18-38 mAu, 230-560 mAu, 560-peak-560 mAu,

560-350 mAu, 130-45 mAu, and 20-10 mAu were collected, and analyzed by SDS-PAGE gel electrophoresis.

[0275] The protein content (Lowry), HPLC, and SDS-PAGE purity of the Heparin chromatographic solution, retentate, SP eluate at 230-560 mAu, SP eluate at 560-peak-560 mAu, and the SP eluate at 560-350 mAu were detected, respectively.

[0276] II. Experimental results

[0277] 1. Results of the influence of the inoculum size of the recombinant engineered bacterium on the rhKGF-2 protein expression level

[0278] When recombinant engineered bacteria with different inoculum sizes were used to produce the rhKGF-2 protein according to the method described in Step 1 of Example 2, the growth curves of the strains are shown in FIG. 14; SDS-PAGE detection results are shown in FIG. 15. Lanes 1 and 6 represent the electrophoresis results of the marker. Lanes 2-5 represent the electrophoresis results of the recombinant engineered bacteria with an 8% inoculum size after fermentation for 2 h, 4 h, 6 h, and 8 h, respectively. Lanes 7-10 are the electrophoresis results of the recombinant engineered bacteria with a 15% inoculum size after 2 h, 4 h, 6 h, and 8 h of fermentation, respectively. The detection results of the plasmid loss rate are shown in Table 4.

Table 4 Detection results of the plasmid loss rate during production of the rhKGF-2 protein by the recombinant engineered bacteria in different inoculum sizes

	Inoculum size of 8%	Inoculum size of 15%
Fermentation for 4 h	6%	6%
Fermentation for 6 h	8%	8%
Fermentation for 8 h	3%	3%

[0279] The results are as follows: During the production of the rhKGF-2 protein, regardless of whether the inoculum size of the recombinant engineered bacterium (500109-S9) is 8% or 15%, there is no significant difference in the growth curves of the bacterium. Moreover, there is no significant difference in the rhKGF-2 protein expression level and the plasmid loss rate. This indicates that increasing the inoculum size of the recombinant engineered bacterium does not improve the rhKGF-2 protein expression level. The

fermentation conditions (temperature, pH, and basal fermentation medium) have already achieved the optimal state for cell growth and rhKGF-2 protein expression. Therefore, an inoculum size of 8% was selected as the optimal inoculum size for the production of the rhKGF-2 protein.

[0280] 2. Results of the influence of the basal fermentation medium on the rhKGF-2 protein expression level

[0281] When different basal fermentation media were used to produce the rhKGF-2 protein according to the method described in Step 1 of Example 2. The growth curves of the strains are shown in FIG. 16; SDS-PAGE detection results are shown in FIG. 17. Lanes 1 and 6 represent the electrophoresis results of the marker; lanes 2-5 represent the electrophoresis results of the recombinant engineered bacteria using the M9Y medium after 2 h, 4 h, 6 h, and 8 h of fermentation, respectively. Lanes 7-10 represent the electrophoresis results of the recombinant engineered bacteria using the basal fermentation medium described in Step 1 of Example 2 after 2 h, 4 h, 6 h, and 8 h of fermentation, respectively. The detection results of the plasmid loss rate are shown in Table 5.

Table 5 Detection results of the plasmid loss rate in the recombinant engineered bacteria producing the rhKGF-2 protein using different basal fermentation media

	The basal fermentation medium is the M9Y medium (F2023042317)	The basal fermentation medium is as shown in Step 1 of Example 2 (F2023042321)
Fermentation for 4 h	2%	4%
Fermentation for 6 h	1%	5%
Fermentation for 8 h	2%	4%

[0282] The results are as follows: During the production of the rhKGF-2 protein, the early growth trend of the recombinant engineered bacteria is obviously poor after replacing the basal fermentation medium shown in Step 1 of Example 2 with the M9Y medium. When the rhKGF-2 protein is produced using two different basal fermentation media in combination with the recombinant engineered bacteria, there is no significant difference in protein expression level. The plasmid loss rate is lower when the M9Y medium is used as the basal

fermentation medium to culture the recombinant engineered bacterium for rhKGF-2 protein production. However, when the M9Y medium is used to culture the engineered bacteria, the growth trend of the strains is obviously inferior to that observed when using the basal fermentation medium shown in Step 1 of Example 2 to culture the engineered bacteria. Therefore, the basal fermentation medium shown in Step 1 of Example 2 was selected for fermentation production of the rhKGF-2 protein in the recombinant engineered bacteria.

[0283] 3. Influence of the inducer addition time on the rhKGF-2 protein expression level

[0284] According to the method shown in Step 1 of Example 2, the inducer (IPTG) was added at different times to produce the rhKGF-2 protein. The growth curve of the strains is shown in FIG. 18; and SDS-PAGE detection results are shown in FIG. 19. Lanes 1-4 represent the electrophoresis results of the recombinant engineered bacteria induced with IPTG at OD_{600} =50 after 2 h, 4 h, 6 h, and 8 h of fermentation, respectively. Lane 5 represents the electrophoresis result of the marker. Lanes 6-9 represent the electrophoresis results of the recombinant engineered bacteria induced with IPTG at OD_{600} =40 after 2 h, 4 h, 6 h, and 8 h of fermentation, respectively. The detection results of the plasmid loss rate are shown in Table 6.

Table 6 Detection results of the plasmid loss rate in the recombinant engineered bacteria for producing the rhKGF-2 protein with IPTG added at different times

	OD_{600} = 50	OD_{600} = 40
Fermentation for 4 h	1%	4%
Fermentation for 6 h	1%	5%
Fermentation for 8 h	6%	4%

[0285] The results are as follows: Regardless of whether the inducer (IPTG) is added at OD_{600} =40 or OD_{600} =50, there is no significant difference in the growth curves of the bacteria. Furthermore, SDS-PAGE detection results indicate that there is no significant difference in protein expression level.

[0286] A higher OD_{600} will cause bacterial cells to be in a stress state, faced with greater metabolic burden, thus breaking metabolic balance, resulting in a decreased growth

rate and the generation of undesirable metabolic products and waste. Meanwhile, a lower OD_{600} may control the rhKGF-2 protein expression level, keeping a low synthesis rate and reducing the occurrence of protein aggregation or degradation. Therefore, it is chosen to add the inducer (IPTG) at OD_{600} =40 for induction.

[0287] 4. Results of the influence of the different inducer concentrations on the rhKGF-2 protein expression level

[0288] According to the method shown in Step 1 of Example 2, different final concentrations of inducer (IPTG) were added to produce the rhKGF-2 protein. The growth curve of the strains is shown in FIG. 20; SDS-PAGE detection results are shown in FIG. 21. Lanes 1 and 6 represent the electrophoresis results of the marker. Lanes 2-5 represent the electrophoresis results of the recombinant engineered bacteria induced with IPTG at a final concentration of 1.5 mM after fermentation for 2 h, 4 h, 6 h, and 8 h, respectively. Lanes 7-10 represent the electrophoresis results of the recombinant engineered bacteria induced with IPTG at a final concentration of 2.0 mM after fermentation for 2 h, 4 h, 6 h, and 8 h, respectively. The detection results of the plasmid loss rate are shown in Table 7.

Table 7 Detection results of the plasmid loss rate in the recombinant engineered bacteria for producing the rhKGF-2 protein with different final concentrations of inducer added

	IPTG = 1.5 mM	IPTG = 2.0 mM
Fermentation for 4 h	2%	4%
Fermentation for 6 h	4%	5%
Fermentation for 8 h	2%	4%

[0289] The results are as follows: The growth trend of the recombinant engineered bacteria increases after changing the final IPTG concentration in Step 1 of Example 2 from 1.5 mM to 2.0 mM. The SDS-PAGE results indicate as follows: Changing the final IPTG concentration from 1.5 mM to 2.0 mM results in a decrease in the rhKGF-2 protein expression level, but the difference is not significant. Changing the final IPTG concentration in Step 1 of Example 2 from 1.5 mM to 2.0 mM also results in a rising trend in the plasmid loss rate during the fermentation production of the rhKGF-2 protein, but the difference is minor. Excessively high concentrations of IPTG will exert toxic effects on cells, causing

inhibition of cell growth or metabolic disorder. Therefore, IPTG at a final concentration of 1.5 mM was selected as the inducer for inducing the recombinant engineered bacteria to produce the rhKGF-2 protein.

[0290] 5. Results of the influence of the different induction temperatures on the rhKGF-2 protein expression level

[0291] According to the method shown in Step 1 of Example 2, the growth curves of the recombinant engineered bacteria for producing the rhKGF-2 protein at different temperatures are shown in FIG. 22. The SDS-PAGE detection results are shown in FIG. 23. Lane 1 represents the electrophoresis result of the marker; lanes 2-5 represent the electrophoresis results of the recombinant engineered bacteria induced at 40°C after fermentation for 2 h, 4 h, 6 h, and 8 h, respectively. Lanes 6-9 represent the electrophoresis results of the recombinant engineered bacteria at the induction temperature of 45°C after fermentation for 2 h, 4 h, 6 h, and 8 h, respectively. The detection results of the plasmid loss rate are shown in Table 8.

Table 8 Detection results of the plasmid loss rate in the recombinant engineered bacteria for producing the rhKGF-2 protein at different induction temperatures

	Induction temperature of 40°C	Induction temperature of 45°C
Fermentation for 4 h	1%	4%
Fermentation for 6 h	1%	5%
Fermentation for 8 h	4%	4%

[0292] The results are as follows: After changing the fermenter temperature in Step 1 of Example 2 to 45°C (i.e., induction temperature of 45°C), the growth trend of the recombinant engineered bacteria during rhKGF-2 protein production declines, and the rhKGF-2 protein expression level decreases significantly.

[0293] Therefore, the induction temperature of 40°C was chosen to induce the recombinant engineered bacteria for fermentation production of the rhKGF-2 protein.

[0294] 6. Results of the influence of the chromatographic column height on the purification effect of the rhKGF-2 protein

[0295] The low-column cation exchange chromatogram is shown in FIG. 24. The high-column cation exchange chromatogram is shown in FIG. 25. The low-column affinity chromatogram is shown in FIG. 26. The high-column affinity chromatogram is shown in FIG. 27.

[0296] The SDS-PAGE detection results for different column heights are shown in FIG. 28. Lane 1 represents the electrophoresis result of the marker. Lane 2 represents the electrophoresis result of the rhKGF-2 protein finely purified at high column height. Lane 3 represents the electrophoresis result of the rhKGF-2 protein finely purified at low column height. The detection results of the rhKGF-2 protein finely purified at different column heights are shown in Table 9.

Table 9 Detection results of the rhKGF-2 protein finely purified at different column heights

Sample	HPLC (%)	SDS-PAGE (%)	Remarks
rhKGF-2 protein finely purified at high column height	96.65	94.0	High column height
rhKGF-2 protein finely purified at a low column height	96.22	92.5	Low column height

[0297] The results are as follows: Increasing the column height of the CM chromatographic column and Heparin chromatographic column in the purification process does not significantly improve the purity of the purified rhKGF-2 protein. This indicates that increasing the column height of the chromatographic columns has little effect on the purification effect of the rhKGF-2 protein.

[0298] 7. Results of the influence of the purification temperature on the purification effect of the rhKGF-2 protein

[0299] The cation exchange chromatogram at a purification temperature of 10°C is shown in FIG. 29. The chromatogram of affinity chromatography at a purification temperature of 10°C is shown in FIG. 30. SDS-PAGE detection results at a purification temperature of 10°C are shown in FIG. 31. Lane 1 represents the electrophoresis result of the marker. Lane 2 represents the electrophoresis result of the rhKGF-2 protein after crude purification at 10°C (i.e., CM elution). Lane 3 represents the electrophoresis result of the

Heparin eluate 1 (80-791-500 mAU) sample; lane 4 represents the electrophoresis result of the Heparin eluate 2 (500-200 mAU) sample; lane 5 represents the electrophoresis result of the Heparin eluate 3 (200-800 mAU) sample; lane 6 represents the electrophoresis result of the rhKGF-2 protein finely purified at a purification temperature of 10°C; and lane 7 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein).

[0300] Detection results of the rhKGF-2 protein crudely and finely purified at a temperature of 10°C are shown in Table 10.

Table 10 Detection results of the rhKGF-2 protein purified at 10°C

Sample	Concentration (mg/ml)	HPLC (%)	SDS-PAGE (%)
rhKGF-2 protein after crude purification at 10°C	7.15	74.27	N/A
rhKGF-2 protein after fine purification at 10°C	2.74	96.57	93.8

[0301] The results are as follows: The SDS-PAGE purity of the rhKGF-2 protein after fine purification at 10°C is 93.8%, and the HPLC purity is 96.57%. Compared with the results of the method shown in Example 2, the purity of the Heparin elution peak (the purified rhKGF-2 protein) remained essentially unchanged. This indicates that controlling the temperature of the Buffer (4-12°C) during the purification chromatography process to control the purification temperature may not have a significant effect on the purification effect of the KGF-2 protein.

[0302] 8. Results of the influence of the impurity washing steps during CM chromatography on the purification effect of the rhKGF-2 protein

[0303] The cation exchange chromatogram after impurity washing is shown in FIG. 32. The chromatogram of affinity chromatography after impurity washing is shown in FIG. 33. SDS-PAGE detection results after impurity washing are shown in FIG. 34. Lane 1 represents the electrophoresis result of the impurity-washed, crudely purified rhKGF-2 protein. Lane 2 represents the electrophoresis result of the marker; lane 3 represents the

electrophoresis result of the impurity-washed, finely purified rhKGF-2 protein. Lane 4 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein).

[0304] Detection results of the impurity-washed, crudely purified rhKGF-2 protein, and the impurity-washed, finely purified rhKGF-2 protein are shown in Table 11.

Table 11 Detection results of the impurity-washed, crudely purified rhKGF-2 protein, and the impurity-washed, finely purified rhKGF-2 protein

Sample	Concentration (mg/ml)	HPLC (%)	SDS-PAGE (%)
Impurity-washed, crudely purified rhKGF-2 protein	5.08	93.59	N/A
Impurity-washed, finely purified rhKGF-2 protein	6.4	95.92	96.1

[0305] The results are as follows: During the CM chromatography process, after adding the impurity washing step (washed with Buffer E), the impurity bands in the crudely purified rhKGF-2 protein reduces obviously, thereby improving the purity of the crudely purified rhKGF-2 protein (CM chromatographic eluate).

[0306] The cation exchange chromatogram after impurity washing with urea is shown in FIG. 35. The chromatogram of affinity chromatography after impurity washing with urea is shown in FIG. 36. SDS-PAGE detection results for Control Group 3 are shown in FIG. 37. Lane 1 represents the electrophoresis result of the marker; lane 2 represents the electrophoresis result of the CM loading sample; lane 3 represents the electrophoresis result of the flow-through solution; lane 4 represents the electrophoresis result of the impurity-washed solution; lane 5 represents the electrophoresis result of the rhKGF-2 protein after impurity washing with urea and crude purification; lane 6 represents the electrophoresis result of the Blank sample; lane 7 represents the electrophoresis result of the rhKGF-2 protein after impurity washing with urea and fine purification; lane 8 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein).

[0307] Detection results of the rhKGF-2 protein after impurity washing with urea and

fine purification are shown in Table 12.

Table 12 Detection results of the rhKGF-2 protein after impurity washing with urea and fine purification

Sample	Concentration (mg/ml)	HPLC (%)	SDS-PAGE (%)
rhKGF-2 protein after impurity washing with urea and fine purification	6.36	97.17	94.2

[0308] The results are as follows: As compared to Experimental Group 2, when the 2 M urea-added Buffer E was used for impurity washing during CM chromatography, the impurity bands and aggregate bands in the crudely purified rhKGF-2 protein reduce (lane 5 in FIG. 37), and the HPLC purity of the purified rhKGF-2 protein reaches 97.17%, with the SDS-PAGE purity of 94.2%.

[0309] Urea, as a denaturant, can change protein hydrophobicity at low concentration, thereby affecting protein-protein interactions. High concentration of urea can break hydrophobic bonds and hydrogen bonds, thus destroying the tertiary and quaternary structures of the protein, thus increasing protein solubility. Therefore, adding urea to Buffer E may disrupt the structure of the rhKGF-2 protein, and the protein purity is not significantly improved. Thus, no urea is added to the impurity-washed solution (Buffer E).

[0310] 9. Results of the influence of the pH during Heparin chromatography on the purification effect of the rhKGF-2 protein

[0311] The cation exchange chromatogram at pH 7.2 is shown in FIG. 38; the chromatogram of affinity chromatography at pH 6.8 is shown in FIG. 39; the chromatogram of affinity chromatography at pH 7.0 is shown in FIG. 40; and the chromatogram of affinity chromatography at pH 7.4 is shown in FIG. 41.

[0312] SDS-PAGE detection results at pH 6.8 are shown in FIG. 42. Lane 1 represents the electrophoresis result of the marker; lane 2 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein). Lane 3 represents the electrophoresis result of the finely purified rhKGF-2 protein during Heparin

chromatography at pH 6.8. Lane 4 represents the electrophoresis result of the Blank (blank control). Lane 5 represents the electrophoresis result of the impurity-washed solution; lane 6 represents the electrophoresis result of the CM loading sample; lane 7 represents the electrophoresis result of the flow-through solution; lane 8 represents the electrophoresis result of the crudely purified rhKGF-2 protein during Heparin chromatography at pH 6.8; and lane 9 represents the electrophoresis result of the Heparin impurity-washed solution.

[0313] SDS-PAGE detection results at pH 7.0 are shown in FIG. 43. Lane 1 represents the electrophoresis result of the marker; lane 2 represents the electrophoresis result of the Heparin impurity-washed solution; lane 3 represents the electrophoresis result of the finely purified rhKGF-2 protein during Heparin chromatography at pH 7.0; lane 4 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein).

[0314] The SDS-PAGE analysis results at pH 7.4 are shown in FIG. 44. Lane 1 represents the electrophoresis result of finely purified rhKGF-2 protein at pH 7.4 during heparin chromatography. Lane 2 represents the electrophoresis result of the heparin elution peak; lane 3 represents the electrophoresis result of the marker, and lane 4 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein).

[0315] Detection results of the finely purified rhKGF-2 protein obtained by heparin chromatography at different pH values are shown in Table 12.

Table 12 Detection results of the finely purified rhKGF-2 protein obtained by heparin chromatography at different pH values

Sample	HPLC (%)	SDS-PAGE (%)	Remarks
Finely purified rhKGF-2 protein at pH 6.8 during heparin chromatography	96.8	91.2	pH 6.8
Finely purified rhKGF-2 protein at pH 7.0 during heparin chromatography	96.3	92.5	pH 7.2
Finely purified rhKGF-2 protein at pH 7.4 during heparin chromatography	96.33	91.9	pH 7.4

Finely purified rhKGF-2 protein at pH 7.2 during heparin chromatography	95.92	96.1	pH 7.2

[0316] The results are as follows: After changing the pH value during heparin chromatography, there is no significant difference in the HPLC purity of the finely purified rhKGF-2 protein. In contrast, when the pH value during heparin chromatography is 7.2, the SDS-PAGE purity of the resulting finely purified rhKGF-2 protein is the highest and significantly higher than other samples. Therefore, the optimal pH value during heparin chromatography is 7.2.

[0317] 10. Results of the influence of the Buffers on the purification effect of the rhKGF-2 protein during heparin chromatography

[0318] The affinity chromatogram of Control Group 7 is shown in FIG. 45; the SDS-PAGE analysis results of the finely purified rhKGF-2 protein from Control Group 7 are shown in FIG. 46. Lane 1 represents the electrophoresis result of the marker; lane 2 represents the electrophoresis result of the heparin elution peak; lane 3 represents the electrophoresis result of the finely purified rhKGF-2 protein from Control Group 7; and lane 4 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein). The detection results of the finely purified rhKGF-2 protein from Control Group 7 are shown in Table 13.

Table 13 Detection results of the finely purified rhKGF-2 protein from Control Group 7

Sample	HPLC (%)	SDS-PAGE (%)
Finely purified rhKGF-2 protein from Control Group 7	98.44	95.7
Finely purified rhKGF-2 protein from Experimental Group 5	98.12	95.6

[0319] The results are as follows: As compared to Experimental Group 5, during heparin chromatography, the Heparin column is equilibrated with 0.9 M NaCl (Buffer C) and the CM chromatographic eluate is directly loaded, which does not result in a significant difference in SDS-PAGE purity. In contrast, when directly loading is performed after column equilibration with 0.9 M NaCl (Buffer C), the protein of interest (rhKGF-2 protein) and

impurity proteins with similar electronegativity to the protein of interest will be bound onto the column simultaneously. Moreover, high concentration of NaCl will weaken the binding between the protein of interest and the chromatographic column, thereby resulting in a low recovery rate.

[0320] The cation exchange chromatogram of Control Group 8 is shown in FIG. 47; the affinity chromatogram of Control Group 8 is shown in FIG. 48; and the SDS-PAGE detection results of Control Group 8 are shown in FIG. 49. Lane 1 represents the electrophoresis result of the marker; lane 2 represents the electrophoresis result of the finely purified rhKGF-2 protein from Control Group 8; lane 3 represents the electrophoresis result of the client stock solution; lane 4 represents the electrophoresis result of the CM loading sample; lane 5 represents the electrophoresis result of the flow-through solution; lane 6 represents the electrophoresis result of the impurity-washed solution; lane 7 represents the electrophoresis result of the crudely purified rhKGF-2 protein from Control Group 8; lane 8 represents the electrophoresis result of the heparin flow-through solution; and lane 9 represents the electrophoresis result of the re-equilibrated heparin flow-through solution. The detection results of the finely purified rhKGF-2 protein from Control Group 8 are shown in Table 14.

Table 14 Detection results of the finely purified rhKGF-2 protein from Control Group 8

Sample	SDS-PAGE (%)	HPLC (%)
Finely purified rhKGF-2 protein from Control Group 8	94.5	95.14

[0321] The results are as follows: After the Heparin chromatographic column is equilibrated with 0.9 M NaCl (Buffer C) and the CM chromatographic eluate is directly loaded, followed by elution with NaCl at a concentration of 0.8 M, the purity of rhKGF-2 protein is not improved. Moreover, the SDS-PAGE detection results show that there are still impurity bands below the finely purified rhKGF-2 protein from Control Group 8. This indicates that elution with Buffer F containing 0.8 M NaCl will not improve the purity of rhKGF-2.

[0322] 11. Results of the influence of the sample loading capacity on the purification

effect of the rhKGF-2 protein during heparin chromatography

[0323] The cation exchange chromatogram of Experimental Group 5 is shown in FIG. 50; the affinity chromatogram of Experimental Group 5 is shown in FIG. 51, the affinity chromatogram of Control Group 9 is shown in FIG. 52, and the affinity chromatogram of Control Group 10 is shown in FIG. 53. The SDS-PAGE detection results at different sample loading capacity are shown in FIG. 54. Lane 1 represents the electrophoresis result of the crudely purified rhKGF-2 protein from Experimental Group 5. Lane 2 shows the electrophoresis result of the finely purified rhKGF-2 protein from Control Group 9. Lane 3 represents the electrophoresis result of the finely purified rhKGF-2 protein from Experimental Group 5. Lane 4 represents the electrophoresis result of the finely purified rhKGF-2 protein from Control Group 10. Lane 5 represents the electrophoresis result of the No. 02 client stock solution, and lane 6 represents the electrophoresis result of the marker.

[0324] The detection results of the finely purified rhKGF-2 protein from Experimental Group 5, the finely purified rhKGF-2 protein from Control Group 9, and the finely purified rhKGF-2 protein from Control Group 10 are shown in Table 15.

Table 15 Protein detection results

Sample	SDS-PAGE (%)	HPLC (%)	Remarks
Finely purified rhKGF-2 protein from Control Group 9	93.7	96.38	0.60 g $_{\text{bacterial sludge}}$/ml $_{\text{filler}}$
Finely purified rhKGF-2 protein from Experimental Group 5	95.5	96.55	1g $_{\text{bacterial sludge}}$/ml $_{\text{filler}}$
Finely purified rhKGF-2 protein from Control Group 10	95.0	96.54	0.90g $_{\text{bacterial sludge}}$/ml $_{\text{filler}}$

[0325] The results are as follows: The sample loading capacity during heparin chromatography has a certain impact on the separation of the finely purified rhKGF-2 protein. When the sample loading capacity during heparin chromatography is 1 g/ml, the purity of the finely purified rhKGF-2 protein is the highest.

[0326] 12. Results of the influence of the elution Buffer during heparin chromatography on the purification effect of the rhKGF-2 protein

[0327] The cation exchange chromatogram of Control Group 11 is shown in FIG. 55; the affinity chromatogram of Control Group 11 is shown in FIG. 56. The SDS-PAGE detection results for different elution buffers are shown in FIG. 57. Lane 1 represents the electrophoresis result of the marker; lanes 2 and 4 both represent the electrophoresis results of the finely purified rhKGF-2 protein from Control Group 11; lane 3 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein); lane 5 represents the electrophoresis result of the CM sample loading sample; lane 6 represents the electrophoresis result of the flow-through solution; lane 7 represents the electrophoresis result of the crudely purified rhKGF-2 protein from Control Group 11; lane 8 represents the electrophoresis result of the impurity-washed fraction; and lane 9 represents the electrophoresis result of the heparin impurity-washed solution.

[0328] The protein detection results of the finely purified rhKGF-2 protein from Control Group 11 are shown in Table 16.

Table 16 Protein detection results of the finely purified rhKGF-2 protein from Control Group 11

Sample	Concentration (mg/ml)	SDS-PAGE (%)	HPLC (%)
Finely purified rhKGF-2 protein from Control Group 11	4.25	95.6	94.12

[0329] The results are as follows: When rhKGF-2 protein is purified according to the method shown in Control Group 11, and Buffer G (i.e., no EDTA-2Na added) is used for heparin chromatographic elution, the HPLC purity of the rhKGF-2 obtained decreases to 94.12% and the SDS-PAGE purity is 95.6%, as compared to the experimental results of Example 2. This indicates that during heparin chromatography elution, adding EDTA-2Na to the buffer may result in higher purity of rhKGF-2.

[0330] 13. Results of the influence of the Buffer pH and addition of Tween 80 during heparin chromatography on the purification effect of the rhKGF-2 protein

[0331] The cation exchange chromatogram of Control Group 12 is shown in FIG. 58; the affinity chromatogram of Control Group 12 is shown in FIG. 59. The SDS-PAGE

detection results for buffers of different pH values are shown in FIG. 60. Lane 1 represents the electrophoresis result of the marker; lane 2 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein); lane 3 represents the electrophoresis result of the finely purified rhKGF-2 protein from Control Group 12; lane 4 represents the electrophoresis result of the CM sample loading sample; lane 5 represents the electrophoresis result of the flow-through solution; lane 6 represents the electrophoresis result of the impurity-washed solution; lane 7 represents the electrophoresis result of the crudely purified rhKGF-2 protein from Control Group 12; and lane 8 represents the electrophoresis result of the heparin impurity-washed solution.

[0332] The protein detection results of the finely purified rhKGF-2 protein from Control Group 12 are shown in Table 17.

Table 17 Protein detection results of the finely purified rhKGF-2 protein from Control Group 12

Sample	Concentration (mg/ml)	HPLC (%)	SDS-PAGE (%)
Finely purified rhKGF-2 protein from Control Group 12	2.01	N/A	83.3

[0333] The results are as follows: When the pH values of Buffer A to Buffer E are adjusted to 7.2 during purification, and Tween 80 is added to Buffer A to Buffer E to a final concentration of 0.05% (w/w), the electrophoresis results show that bands of the protein of interest are degraded and the impurity protein bands are significantly deepened. Furthermore, the purity of the obtained rhKGF-2 protein decreases significantly, and the HPLC detection results are abnormal. This indicates that Tween 80 is not suitable for all protein purification processes; adding Tween-80 to the purification buffer and adjusting the pH value will significantly decrease the final purification effect.

[0334] 14. Results of the influence of post-chromatography membrane exchange on the purification effect of the rhKGF-2 protein

[0335] The cation exchange chromatogram of Experimental Group 7 is shown in FIG. 61; the affinity chromatogram of Experimental Group 7 is shown in FIG. 62. The SDS-PAGE

detection results for the addition of membrane exchange after chromatography are shown in FIG. 63. Lane 1 represents the electrophoresis result of the crudely purified rhKGF-2 protein from Experimental Group 7; lane 2 represents the electrophoresis result of the marker; lane 3 represents the electrophoresis result of the finely purified rhKGF-2 protein from Experimental Group 7; lane 4 represents the electrophoresis result of the KGF-2 stock solution (the sample containing only the KGF-2 protein); lane 5 represents the electrophoresis result of the finely purified rhKGF-2 protein from Control Group 14; lane 6 represents the electrophoresis result of the dialysate from Control Group 14; lane 7 represents the electrophoresis result of the finely purified rhKGF-2 protein from Control Group 13; and lane 8 represents the electrophoresis result of the dialysate from Control Group 13.

[0336] Protein detection results are shown in Table 18.

Table 18 Protein detection results

Sample	Concentration (mg/ml)	HPLC (%)	SDS-PAGE (%)	Remarks
Finely purified rhKGF-2 protein from Experimental Group 7	6.4	95.92	96.1	N/A
Finely purified rhKGF-2 protein from Control Group 13	6.20	96.26	96.9	N/A
Dialysate from Control Group 13	0	N/A	N/A	No protein permeation observed
Finely purified rhKGF-2 protein from Control Group 14	0.84	95.10	95.6	N/A
Dialysate from Control Group 14	1.84	N/A	N/A	Protein permeation observed

[0337] The results are as follows: As compared to Experimental Group 7, when Buffer H without NaCl and EDTA serves as the membrane exchange solution for dialysis with a 2KD dialysis membrane, there is no significant difference in concentration, HPLC

purity, or SDS-PAGE purity of the finely purified rhKGF-2 protein obtained in Control Group 13. However, after replacing the 2KD dialysis membrane with a 5KD dialysis membrane, the rhKGF-2 protein will permeate through the 5KD membrane, causing a significant decrease in the concentration of the finely purified rhKGF-2 protein obtained in Control Group 14. Therefore, dialysis membrane exchange is not necessary after heparin chromatography.

[0338] 15. Effect of the rhKGF-2 protein concentration on stability

[0339] The cation exchange chromatogram of Experimental Group 8 is shown in FIG. 64; the affinity chromatogram of Experimental Group 8 is shown in FIG. 65. The SDS-PAGE detection results for different concentrations of rhKGF-2 protein are shown in FIG. 66. Lane 1 represents the electrophoresis result of the marker; lane 2 represents the electrophoresis result of the blank; lane 3 represents the electrophoresis result of the client stock solution; lane 4 represents the electrophoresis result of the 3.3 mg/mL diluted solution; lane 5 represents the electrophoresis result of the 3.0 mg/mL diluted solution; lane 6 represents the electrophoresis result of the 2.0 mg/mL diluted solution; and lane 7 represents the electrophoresis result of the 1.0 mg/mL diluted solution.

[0340] The protein detection results for the diluted solution of different concentrations are shown in Table 19.

Table 19 Protein detection results for the diluted solution of different concentrations

Sample	HPLC (%)	SDS-PAGE (%)
3.3 mg/ml diluted solution	95.48	94.2
3.0 mg/ml diluted solution	96.20	93.2
2.0 mg/ml diluted solution	95.71	95.6
1.0 mg/ml diluted solution	94.69	95.7
0.5 mg/ml diluted solution	92.98	98.2

[0341] The results are as follows: The lower the rhKGF-2 protein concentration in the diluted solution is, the lighter the KGF-2 protein bands and impurity protein bands in the SDS-PAGE results are, and the lower the HPLC purity is. Excessively high rhKGF-2 protein concentration will lead to protein aggregation.

[0342] Therefore, when the rhKGF-2 protein concentration is controlled at 2.0 mg/mL, the SDS-PAGE and HPLC purity of the rhKGF-2 protein are higher.

[0343] 16. Effect of adding SP chromatography after heparin chromatography on the purification effect of the rhKGF-2 protein

[0344] The cation exchange chromatogram of Experimental Group 9 is shown in FIG. 67; the affinity chromatogram of Experimental Group 9 is shown in FIG. 68, and the SP chromatogram of Experimental Group 9 is shown in FIG. 69. The SDS-PAGE electrophoretograms of the heparin chromatographic eluate and retentate from Experimental Group 9 are shown in FIG. 70. Lane 1 represents the electrophoresis result of the heparin chromatographic eluate; lane 2 represents the electrophoresis result of the retentate. The SDS-PAGE electrophoresis results of the SP eluate from Experimental Group 9 are shown in FIG. 71. Lane 1 represents the marker; lane 2 represents the client 02 stock solution; lane 3 represents the SP eluate at 5-10 mAu; lane 4 represents the SP eluate at 18-38 mAu; lane 5 represents the SP eluate at 230-560 mAu; lane 6 represents the SP eluate at 560-peak-560 mAu; lane 7 represents the SP eluate at 560-350 mAu; lane 8 represents the SP eluate at 130-45 mAu; and lane 9 represents the SP eluate at 20-10 mAu.

[0345] The protein detection results of Experimental Group 9 are shown in Table 20.

Table 20 Protein detection results of Experimental Group 9

Sample	Concentration (mg/ml)	HPLC (%)	SDS-PAGE (%)
Heparin chromatographic eluate	4.56	96.91	93.8
Retentate	2.9	97.82	94.4
SP eluate at 230-560 mAu	1.88	96.88	93
SP eluate at 560-peak-560 mAu	3.09	97.94	94.4
SP eluate at 560-350 mAu	2.73	98.50	95.3

[0346] The results are as follows: When SP chromatography is added after CM chromatography and heparin chromatography, bands of the protein of interest (KGF-2 protein) may be not further separated from the impurity bands. It fails to further effectively improve the purity of the KGF-2 protein.

[0347] Example 4 Process scale-up of the method for producing and purifying the rhKGF-2 protein

[0348] 1. Experimental method

[0349] (1) 1 L process scale-up for production and purification of the rhKGF-2 protein

[0350] Fermentation was performed according to the method shown in S1-S3 of Step 1 in Example 2, to obtain the fermented engineered bacteria.

[0351] Buffer A to Buffer E for column chromatography were prepared according to S4 of Step 1 in Example 2, and the pH values of Buffer A to Buffer E were adjusted to 7.2.

[0352] The fermented engineered bacteria were mixed with Buffer A at a ratio of 1 g:20 mL, stirred thoroughly to obtain a mixed solution. At 15°C, the mixed solution was homogenized and disrupted once at 200 Bar first, and then homogenized and disrupted twice at 700 Bar. After complete disruption (i.e., no intact bacterial cells in the mixed solution, OD600 < 30), the homogenized liquid was collected and centrifuged at 7,000 rpm, 10°C for 20 min. The supernatant was collected and filtered, and the filtrate was collected.

[0353] A CM chromatographic column (Borglon CM Bestarose Fast Flow, CV:1.4L) was connected and equilibrated with Buffer A at a linear velocity of 100 cm/h for 3 column volumes until the CM chromatography column baseline was stable and the conductivity was ≤100ms/cm. The effluent pH of the CM chromatography column was 7.2, and the UV_{280} absorption baseline was stable. The filtrate (CM sample loading sample) was loaded at 0.8 g/mL, and the flow-through solution was collected. Buffer A was used to re-equilibrate the CM chromatography column until the UV_{280} absorption baseline was stable. Next, the impurity-washed solution (Buffer E) was used to wash 2 column volumes, collection started when UV > 20 mAU and stopped when UV < 20 mAU, to obtain the wash fraction. Buffer C was then used for elution of 2 column volumes; collection started when UV_{280} >100 mAU and stopped when UV_{280} <100 mAU. The CM chromatographic eluate was collected, namely, the crudely purified rhKGF-2 protein. After elution, the CM chromatographic column was regenerated, cleaned, and stored.

[0354] A Heparin chromatographic column (Borglon Heparin Bestarose Fast Flow, CV:450mL) was connected and equilibrated with Buffer B at a linear velocity of 60cm/h for 3

column volumes until the Heparin chromatography column baseline was stable and the conductivity was ≤ 100 ms/cm. The effluent pH of the heparin chromatography column was 7.2, and the UV_{280} absorption baseline was stable. The CM chromatographic eluate was mixed with an equal volume of Buffer A and loaded at 1 g/mL, and the flow-through peak was collected. Buffer B was used to re-equilibrate the Heparin chromatographic column until the UV_{280} absorption baseline was stable. Next, Buffer C was used to wash impurities, and after the UV_{280} absorption baseline was stabilized via washing, the heparin impurity-washed solution was collected. After washing, Buffer D was used for elution of 2 column volumes; collection started when UV_{280} >100 mAU and stopped when UV_{280} < 100 mAU, to obtain the Heparin chromatographic eluate, namely, the finely purified rhKGF-2. After elution, the Heparin chromatographic column was regenerated, cleaned, and stored.

[0355] Buffer H (pH=7.2, 30 mM PB) was used as the membrane exchange solution, mixed with the finely purified rhKGF-2 at equal volume, and exchanged three times with a 2KD dialysis membrane. The rhKGF-2 retentate was collected.

[0356] (2) Protein expression detection

[0357] The finely purified rhKGF-2, rhKGF-2 dialysate, and KGF-2 stock solution (the sample containing only the KGF-2 protein) were subjected to SDS-PAGE gel electrophoresis detection; and rhKGF-2 retentate was subjected to HPLC assay and protein content assay.

[0358] 2. Experimental results

[0359] The SDS-PAGE gel electrophoresis results are shown in FIG. 72. Lane 1 represents the marker; lane 2 represents the blank lane; lane 3 represents the rhKGF-2 retentate; lane 4 represents the finely purified rhKGF-2; and lane 5 represents the KGF-2 stock solution (the sample containing only the KGF-2 protein). The detection results for rhKGF-2 retentate are shown in Table 21.

Table 21 Detection results for the rhKGF-2 retentate

Sample	Concentration (mg/mL)	HPLC (%)	SDS-PAGE (%)
rhKGF-2 retentate	3.32	94.74	96.40

[0360] The results are as follows: During the 1 L process scaling up for production of the rhKGF-2 protein, the HPLC purity and SDS-PAGE purity of the obtained rhKGF-2 protein are not significantly different from those of the rhKGF-2 protein produced by the method described in Example 2. This indicates that the method for producing the rhKGF-2 protein shown in Example 2 is suitable for process scale-up.

[0361] **Comparative Example 1 Expression and purification of the recombinant human KGF-2 protein**

[0362] 1. Experimental method

[0363] The fermented engineered bacteria were obtained according to steps S1-S3 of Example 2, and Buffer A-Buffer E for column chromatography were prepared as described in step S4.

[0364] The fermented engineered bacteria were mixed with Buffer A at a ratio of 1 g:20 mL, stirred thoroughly to obtain a mixed solution. At 15°C, the mixed solution was homogenized and disrupted once at 200 Bar first, and then homogenized and disrupted twice at 700 Bar. After complete disruption (i.e., no intact bacterial cells in the mixed solution, $OD_{600} < 30$), the homogenized liquid was collected and centrifuged at 7,000 rpm, 10°C for 20 min. The supernatant was collected and filtered, and the filtrate was collected.

[0365] CM chromatography: The CM chromatographic column (Cytiva CM Sepharose Fast Flow, CV:70 mL) was connected and equilibrated with Buffer A at a linear velocity of 60 cm/h for 3 column volumes until the CM chromatographic column baseline was stable and the conductivity was ≤100 ms/cm. The effluent pH of the CM chromatographic column was 7.2, and the UV_{280} absorption baseline was stable. The filtrate (CM sample loading sample) was loaded at 0.5 g/mL, and the flow-through solution was collected. Buffer A was used to re-equilibrate the CM chromatographic column until the UV_{280} absorption baseline was stable. Next, Buffer C was used to elute 2 column volumes. Collection of the eluate started when $UV_{280} > 50$ mAU and stopped when $UV_{280} < 50$ mAU. The CM chromatographic eluate was collected, namely, the crudely purified rhKGF-2 protein. Moreover, the CM chromatographic column was regenerated, cleaned, and stored.

[0366] Heparin chromatography: The Heparin chromatographic column (Cytiva Heparin Sepharose Fast Flow, CV: 40 mL) was collected, and equilibrated with Buffer B at a

linear velocity of 60 cm/h for 3 column volumes until the baseline of the Heparin chromatographic column was stable and the conductivity was ≤ 100 ms/cm, the effluent of the Heparin chromatographic column had a pH value of 7.2 and a stable UV_{280} absorption baseline. The CM chromatographic eluate was mixed with Buffer A in equal volumes, and loaded at 0.5 g/mL, and then the flow-through peak was collected. Then, the Heparin chromatographic column was re-equilibrated with Buffer B until the UV_{280} absorption baseline was stable. Next, impurities were washed off with Buffer C, and after the UV_{280} absorption baseline was stable via washing, the heparin impurity-washed solution was collected. After impurity washing, the column was eluted with Buffer D for 2 column volumes. Collection of the eluate started when UV_{280} >80 mAU, and stopped when UV_{280} < 80 mAU, to obtain Heparin chromatographic eluate (heparin eluate), namely, the finely purified rhKGF-2 from Comparative Example 1. The Heparin chromatographic column was regenerated, cleaned, and stored.

[0367] According to the method described in step 2 of Example 2, the protein content and purity (HPLC and SDS-PAGE) of the finely purified rhKGF-2 from Comparative Example 1 were measured.

[0368] 2. Experimental results

[0369] The detection results of the expression level of the finely purified rhKGF-2 protein from Comparative Example 1 are shown in Table 22.

Table 22 Detection results of the expression level of the finely purified rhKGF-2 protein from Comparative Example 1

	HPLC (%)	SDS-PAGE (%)
Finely purified rhKGF-2 protein from Comparative Example 1	96.65	92.5

[0370] The SDS-PAGE results of the finely purified rhKGF-2 protein from Comparative Example 1 are shown in FIG. 73. The results are as follows: In the protein graph (SDS-PAGE result) of the finely purified rhKGF-2 from Comparative Example 1, there are several thin impurity protein bands below the band of the protein of interest, and the integration shows that the purity of rhKGF-2 is less than 95%.

[0371] It should be finally noted that the above examples are only intended to illustrate the technical solutions of the present invention but are not construed as limiting the scope of protection of the present invention. For those skilled in the art, other different forms of variations or modifications may be made based on the above description and ideas, and it is neither necessary nor possible to exhaust all embodiments here. Any modifications, equivalent substitutions, and improvements within the spirit and principles of the present invention shall be included within the scope of protection of the claims of the present invention.

WHAT IS CLAIMED IS:

1. An engineered bacterium for producing a KGF-2 protein, wherein the engineered bacterium is *Escherichia coli* transfected with a recombinant vector;

the recombinant vector is a pET-30a vector carrying a KGF-2 protein coding sequence, a pET-28a vector carrying a KGF-2 protein coding sequence, or a pET-42a vector carrying a KGF-2 protein coding sequence, and the KGF-2 protein has an amino acid sequence as shown in SEQ ID NO: 2; and the *Escherichia coli* is OverExpress C43(DE3) *Escherichia coli*, Rosetta(DE3) *Escherichia coli*, or BL21 Star(DE3) *Escherichia coli*.

2. The engineered bacterium according to claim 1, wherein the recombinant vector is the pET-30a vector carrying the KGF-2 protein coding sequence, and the *Escherichia coli* is Rosetta(DE3) *Escherichia coli* or OverExpress C43(DE3) *Escherichia coli*.

3. Use of the engineered bacterium according to any one of claims 1-2 in producing a KGF-2 protein.

4. A method for producing a KGF-2 protein, wherein the KGF-2 protein is produced by fermenting the engineered bacterium according to any one of claims 1-2.

5. The method according to claim 4, wherein the method comprises the following steps:

step S1, inoculating the engineered bacteriumany one of claims 1-2 into an LB medium in a volume ratio of 1:20-200, adequately culturing to obtain a primary seed solution, and inoculating the primary seed solution into the LB medium in a volume ratio of 1:20-200, and culturing adequately to obtain a secondary seed culture solution;

step S2, mixing the secondary seed culture solution obtained in the step S1 with a basal fermentation medium in a volume ratio of 2-10:20-100 to obtain a culture solution; adjusting a pH value of the culture solution to 6.2-8.2, performing culturing under conditions of a dissolved oxygen of greater than 10%, a temperature of 25-45°C, a rotating speed of 100-1,600 rpm, and an aeration rate of 5-100 L/min, and when an OD_{600} of the culture solution reaches 10-50, adding IPTG at a final concentration of 0.1-3.0 mM and a feed solution accounting for 1.0-30% of a total volume to the culture solution, and performing adequate culture to obtain a fermented engineered bacterial cell; wherein

the basal fermentation medium is obtained by mixing a basal medium 1, a basal medium 2, and 1-20 g/L of a thiamine solution in a ratio of 15-25 L:0.5-1.5 L:6-10 mL;

the basal medium 1 comprises: at final concentrations, 15-25 g/L peptone, 15-25 g/L yeast powder, 3-7 g/L sodium chloride, 1-3 g/L potassium dihydrogen phosphate, 2-4 g/L dipotassium hydrogen phosphate, and 1-3 g/L ammonium chloride; and the basal medium 2 comprises, at final concentrations, 2-10 g/L glucose monohydrate, 0.2-0.8 g/L anhydrous magnesium sulfate, and 0.01-0.09 g/L calcium chloride;

the feed solution comprises: at final concentrations, 15-25 g/L peptone, 15-25 g/L yeast powder, 3-7 g/L sodium chloride, 1-3 g/L potassium dihydrogen phosphate, and 2-4 g/L dipotassium hydrogen phosphate trihydrate; and

step S3, homogenizing and disrupting the fermented engineered bacterial cell obtained in the step S2, performing solid-liquid separation to collect a supernatant for CM chromatography to obtain a crudely purified KGF-2 protein, and performing Heparin chromatography on the crudely purified KGF-2 protein, to obtain the KGF-2 protein.

6. The method according to claim 5, wherein the LB medium in the step S1 is an LB medium comprising, at final concentrations, 2-10 g/L yeast extract powder, 9-21 g/L peptone, and 9-21 g/L sodium chloride, with a pH value of 6.2-8.2.

7. The method according to claim 5, wherein in the step S2: when the OD_{600} of the culture solution reaches 40, IPTG at a final concentration of 0.1-3.0 mM and the feed solution accounting for 1.0-30% of the total volume are added to the culture solution.

8. The method according to claim 5, wherein in the step S3, the homogenizing and disrupting is specifically as follows: fully mixing the fermented engineered bacterial cell obtained in the step S2 with Buffer A in a ratio of 1 g:1-100 mL for homogenization and disruption at 4-40°C; and

the Buffer A has a formulation of: 15-45 mM phosphate buffer, 0.2-1.8 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2.

9. The method according to claim 5, wherein the CM chromatography in the step S3 is specifically as follows:

equilibrating a CM chromatographic column, adding the supernatant obtained in the step S3 at 0.01-10.0 g/mL to the CM chromatography column equilibrated, collecting a flow-through peak, re-equilibrating the CM chromatographic column with Buffer A, washing off impurities thoroughly with Buffer E, and then eluting with Buffer C for 0.5-10 column

volumes, and collecting an eluate when UV_{280} > 20 mAU, to obtain the crudely purified KGF-2 protein;

the Buffer C has a formulation of: 15-45 mM phosphate buffer, 0.2-1.8 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2; and

the Buffer E has a formulation of: 15-45 mM phosphate buffer, 0.2-1.8 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2.

10. The method according to claim 5, wherein the Heparin chromatography in the step S3 comprises the following steps:

equilibrating a Heparin chromatographic column, mixing the crudely purified KGF-2 protein in the step S3 with an equal volume of Buffer A, loading the Heparin chromatographic column equilibrated at 0.10-2.00 g/mL, collecting a flow-through peak, re-equilibrating the Heparin chromatographic column with Buffer B, washing off impurities thoroughly with Buffer C, then eluting the column with Buffer D for 0.5-10 column volumes, and collecting an eluate when UV_{280} > 20 mAU, to obtain the KGF-2 protein; wherein

the Buffer B has a formulation of: 15-45 mM phosphate buffer, 0.1-3 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2; and

the Buffer D has a formulation of: 15-45 mM phosphate buffer, 0.1-3 M sodium chloride, and 5-35 mM EDTA-2Na, with a pH value of 6.2-8.2.