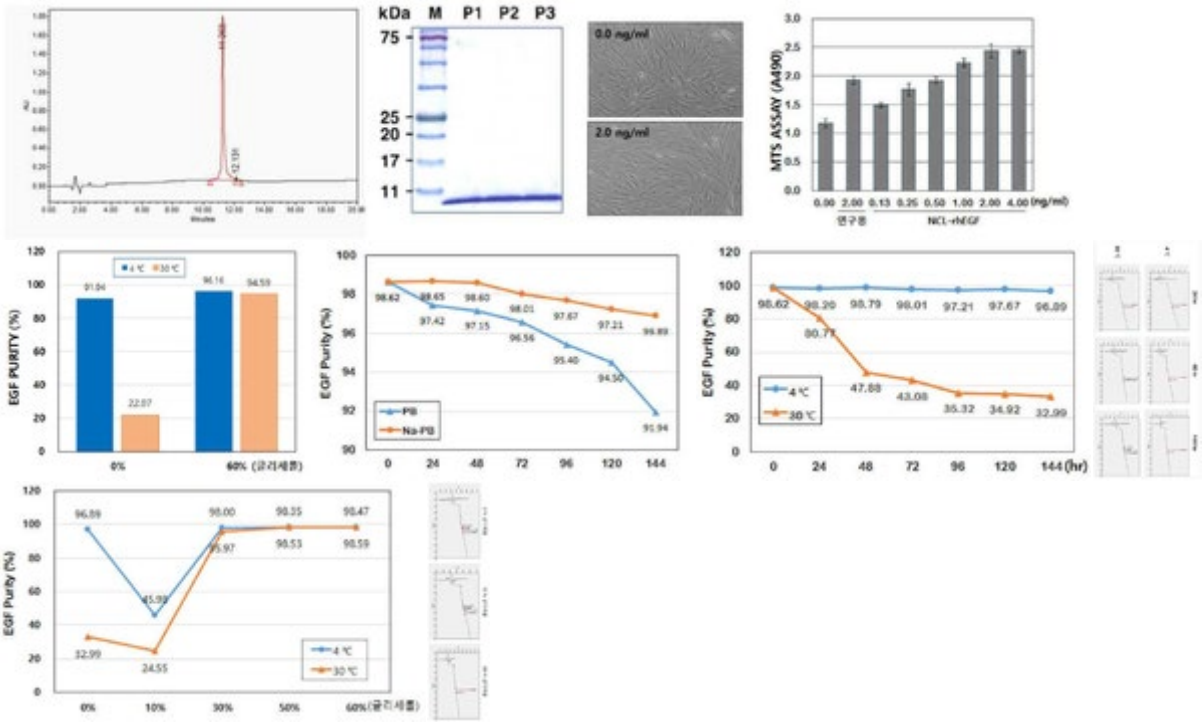
The present invention relates to compositions of human epidermal growth factor (EGF) solutions that promote thermal stabilization. The present invention also relates to a cosmetic and pharmaceutical composition which is excellent in heat stability to a solution during distribution and storage through use of a composition for promoting the thermal stabilization of human epithelial cell growth factor and maintains its activity.

Images (8)



Classifications

A61K8/64 Proteins; Peptides; Derivatives or degradation products thereof

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Description

translated from Korean

[0001] The present invention relates to compositions of human epidermal growth factor

The present invention relates to a composition of a solution containing human Epidermal Growth Factor (EGF) which promotes thermal stability with temperature change. More particularly, the present invention relates to a composition of EGF which improves thermal stability by glycerin, sodium chloride, or an acidic buffer solution, and its use.

The pharmaceutical industry is largely classified as synthetic medicine and biopharmaceutical industry, and the biopharmaceutical industry using biotechnology has a great growth potential and is attracting

attention as a high value-added industry. The mainstream of these biopharmaceuticals is protein drugs, which are genetically engineered to produce recombinant proteins using microorganisms and animal cells. These recombinant proteins (native forms) have the same tertiary structure and biological activity as the biologically derived proteins Do.

These recombinant (natural) proteins have been physically and chemically unstable to heat, making it difficult to produce, distribute and store protein drugs. As a biobeta, there is a lot of research and investment in improving the thermostability of proteins and maximizing their efficacy.

As one of the bio-derived proteins, human epithelial cell growth factor (EGF) is a 6,0452 dalton-sized protein composed of 53 amino acids with three disulfide bonds (-S-S-bond). In vivo, EGF has biological activity such as promotion of cell mitosis, promotion of cell growth, inhibition of gastric acid secretion, and is currently being used as a treatment for wound healing, damage to gastric wall, and treatment of foot ulcers in diabetic patients. Currently, it is listed in the International Cosmetic Ingredient Collection (ICID) of the American Cosmetics Association (CTFA) because of its biological activity on epithelium and skin cells. It has been approved as a raw material for cosmetics by the Food and Drug Administration.

Recently, it has become possible to mass-produce pure human EGF by using a genetic engineering technique to transform a human EGF gene into a microorganism to produce the same protein as that of human EGF, and to separate and purify the human EGF gene. However, the produced human EGF is sensitive to temperature and, when contained in a relatively high temperature aqueous solution, the biological activity is rapidly reduced due to a rapid tertiary structure change.

On the other hand, proteins are easily denatured by minute environmental changes such as temperature, pressure, pH and ion concentration. Factors influencing protein denaturation include charge transfer by strong acid and strong alkali, SS bond breakdown by reducing agent, protein aggregation by salt concentration, destruction of H-bond and electrostatic attraction due to increase of protein molecular motion by temperature change , And weak bond failure due to mechanical stress. In order to maintain the structure of a specific protein having biological activity, stabilization of the protein is required for various environmental conditions. Therefore, thermal stabilization of the protein is a very important factor for commercialization of these proteins.

Generally, methods for stabilizing proteins include screening and setting optimal temperature and optimum ion concentration according to the factors described above, chemically modifying proteins, protein engineering methods, immobilization methods, or addition of stabilizers Method. Stabilizers used to increase the thermal stability of proteins include polyols, dextran, bovine serum albumin, polyethylenimine, polyelectrolytes, organic osmolytes, organic solvents, sugars and other substances Are illustrated.

The selection and concentration of these stabilizers must be determined experimentally according to the physicochemical properties of the tertiary structure of the protein to be thermally stabilized, and the kind and concentration of the stabilizer determined in one protein It is not universally applicable.

Therefore, in order to use human EGF as a therapeutic agent for medicines and raw materials for cosmetics, techniques for maintaining the original structure and activity of human EGF at a room temperature including a low temperature and a relatively high temperature are urgently needed. Therefore, it is very important to develop a composition that increases the thermal stability of human EGF by using a composition prepared by adding a substance that does not affect the human body to an aqueous solution of human EGF.

As a prior art relating to thermal stabilization of human epithelial cell growth factor, Korean Patent Publication No. 10-1996-0013439 discloses a stable epithelial growth factor composition. Here, it is

proposed to use biologically, physically and chemically stable EGF composition as an eye drop and topical ointment, while biological activity is maintained by adding an additive selected from phenol, polyethylene glycol, fatty acid and the like to the epithelial growth factor. However, the additives used here are limited in their purity depending on the phenol which is harmful to the human body.

Korean Patent Laid-Open Publication No. 10-2010-0002388 discloses a surfactant-type peptide for improving the stability of epithelial growth factor and its use. Since the surfactant-type peptide used here is composed of a specific amino acid sequence, it is difficult to produce the peptide easily, and thus its use has been limited.

Furthermore, Korean Patent Publication No. 10-1519118 discloses a high-stability epithelial growth factor variant. Here, since the amino acid sequence (protein primary structure) in the EGF is modified using genetic engineering techniques, the EGF mutant is improved to improve the thermal stability, so that it is distinguished from the composition for enhancing the thermal stability by the additive.

It is an object of the present invention to provide a pharmaceutical composition which is superior in thermal stability to phenol, polyethylene glycol, fatty acid, or surfactant-type peptide known in the prior art, and which has no physicochemical properties and has no harmful effects on the human body. , And to provide a composition for cosmetics and pharmaceuticals using the same.

It is an object of the present invention to provide a pharmaceutical composition containing EGF in which a solution containing human epithelial cell growth factor (EGF) is added with glycerol at a weight ratio (w / v) of 10 to 50% Can be achieved.

In one embodiment of the present invention, the solution containing the EGF composition of the present invention is a sodium phosphate solution, and the concentration of EGF in the sodium phosphate solution is in the range of 0.1 μg / ml to 1,000 μg / ml.

In another embodiment of the present invention, the EGF composition of the present invention further comprises sodium chloride in an amount of 10 mM to 100 mM.

In another embodiment of the present invention, the EGF composition of the present invention further comprises an acidic buffer solution for adjusting the pH of the composition to 4 to 8, in an amount of 10 mM to 100 mM. The acidic buffer solution used herein is preferably a sodium phosphate solution or a citric acid solution.

In another aspect of the present invention, there is provided a cosmetic composition of EGF comprising a cosmetically effective amount of the above-mentioned composition of EGF and a cosmetically acceptable carrier.

In another aspect of the present invention, the present invention provides a composition for treating skin wounds of EGF comprising a pharmaceutically effective amount of the composition of EGF described above and a pharmaceutically acceptable carrier.

The composition of human EGF in the aqueous solution state prepared according to the present invention can maintain long-term thermal stability even at a low temperature of 4 캜 and a high temperature of 30 캜. Accordingly, since the EGF product using the human EGF composition of the present invention is stable during distribution and storage, functional cosmetics and medicines that maintain the natural EGF activity will be made possible.

1 shows the purity and structure of human EGF prepared by the present applicant (New Cell Life Co., Ltd.) directly by HPLC and SDS-PAGE.
FIG. 2 is a comparative analysis of the biological activity of human EGF produced with human EGF for study as human normal skin cell (HDF) proliferation rate.
FIG. 3 shows the results of the finding that the EGF composition containing glycerol has an excellent effect as a thermal stabilizer of the EGF tertiary structure.
Figure 4 shows the results of the finding that an EGF composition comprising sodium chloride functions as a stabilizer to stabilize human EGF.
FIG. 5 shows the results of analysis of the tertiary structure change of human EGF over time at 30 ° C.
Figure 6 shows HPLC chromatograms for structural changes of human EGF at 4 < 0 > C and 30 < 0 > C temperature and time.
FIG. 7 shows the results of analysis of the activity as a heat stabilizer for stabilizing the human EGF tertiary structure by temperature and time in an EGF composition containing glycerol and sodium chloride.
8 is an HPLC chromatogram showing the results of the change of the EGF tertiary structure (red line portion) by glycerol concentration when stored at 30 DEG C for 144 hours.

The present invention provides a composition of EGF in which a solution containing human epithelial cell growth factor (EGF) is added with glycerol at a weight ratio (w / v) of a solution of EGF at 10% to 50% to increase thermal stability.

EGF used in the present invention is either dissolved in a phosphate buffer solution or treated as a lyophilized powder and is commercially available. EFG in a lyophilized powder state hardly causes a problem of thermal stability, and a problem of thermal stability will occur in an EGF solution which is mainly dissolved in a buffer solution. The concentration of EGF in the sodium phosphate solution used as the buffer is present in an amount of 0.1 μg / ml to 1,000 μg / ml, preferably 0.1 μg / ml to 100 μg / ml.

In addition, although the purity of EGF commercially available on the market is usually about 95%, the EGF used in the present invention has a purity of about 98% by further purification and separation in order to clearly confirm the effect of the present invention Will be used. The phosphate solvent in which the EFG is dissolved is not limited unless the chemical nature of the EGF is modified, but a sodium phosphate solvent can be preferably used.

In the present invention, the biological activity of EGF refers to the proliferative activity of human dermal fibroblast (HDF) commercially available for research, and the proliferative activity of EGF was quantitatively analyzed by MTS assay. In general, the activity of EGF is analyzed using mouse-derived NIH3T3 or Balb / c 3T3 cell line transformed cells. In the present invention, more accurate EGF biological activities applicable to human body by using human-derived normal skin cells We have provided a method to analyze.

The glycerol used in the present invention is a colorless and odorless liquid, also called glycerin, which is harmless to the human body and is generally used in pharmaceuticals and cosmetics. This is a fat component as well as a fatty acid, and is obtained industrially by decomposing fat. Glycerin is a colorless, persistent liquid that is hygroscopic and has a little sweetness, and is generally used in reagent grade research, cosmetics (moisturizers) and medicines (enema). However, the glycerol used in the present invention is used as an EGF thermal stabilizer regardless of this generally known use.

Such glycerol is used in the range of 10% to 50% in terms of the weight ratio (w / v) of the solution of EGF used in the present invention. If it is less than 10% in this range, the effect is lowered for the intended purpose of increasing the thermal stability. If it exceeds 50%, the effect is the same as 50% for the purpose of use. It is not easy to do.

As used herein, the term " thermal stability " means stability with respect to the tertiary structural modification of EGF according to a change in temperature, and not only stability in vivo but also storage stability (for example, Storage stability).

In particular, the term " tertiary structure " of protein and EGF in the present specification refers to the sequence or sequence (primary structure) of the amino acids constituting the protein and EGF and the hydrogen bond formed between the peptide bond of the amino acid bond in the primary structure Bond) and an amino acid residue (R group) bond (for example, -SS-; disulfide bond). The tertiary structure of EGF-containing proteins exhibits unique physico-chemical properties. When the tertiary structure is modified, the physicochemical properties such as hydrophilicity are changed, resulting in degradation or loss of biological activity.

In the present invention, the modification of the tertiary structure was analyzed by the change of retention time (RT) in HPLC since the HPLC can analyze the minute change according to the modification of the tertiary structure.

In another embodiment, the composition of EGF of the present invention may further comprise sodium chloride, and the amount thereof is in the range of 10 mM to 100 mM, preferably in the range of 10 mM to 50 mM. If the concentration is less than 10 mM, the effect of increasing the thermal stability is lowered. If the concentration is more than 100 mM, the use effect of increasing the thermal stability is lowered.

As another embodiment, the composition of EGF of the present invention may further comprise an acidic buffer solution. By using such a buffer solution, the pH of the composition of EGF of the present invention can be adjusted to a range of 5.6 to 7.0 have.

The amount thereof used for controlling the acidity is in the range of 10 mM to 100 mM, preferably in the range of 10 mM to 50 mM. If the concentration is less than 10 mM, the effect of increasing the thermal stability is lowered. If the concentration is more than 100 mM, the use effect of increasing the thermal stability is lowered.

The acidic buffer solution used in the present invention may be any of those generally used in cosmetic or pharmaceutical compositions, but is preferably a sodium phosphate solution or a citric acid solution.

In another aspect, the present invention provides a cosmetic composition of EGF comprising a cosmetically effective amount of a composition of EGF and a cosmetically acceptable carrier.

As used herein, "a cosmetically effective amount" means an amount sufficient to achieve the skin-improving effect of the composition of the present invention described above. The cosmetic composition of the present invention may be formulated into any formulation conventionally produced in the art and may be formulated into, for example, solutions, suspensions, emulsions, pastes, gels, creams, lotions, , But is not limited thereto.

Although the carrier used in the cosmetic composition of the present invention varies slightly depending on the above-mentioned formulations, for example, when the formulation is a solution or an emulsion, a solvent, a dissolving agent or an emulsifying agent is used as a carrier component, Examples thereof include fatty acid esters of water, ethanol, isopropanol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butyl glycol oil, glycerol aliphatic ester, polyethylene glycol or sorbitan.

In another aspect, the invention provides a composition for treating skin or improving wound healing of EGF comprising a pharmaceutically effective amount of a composition of EGF and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically effective amount" means an amount sufficient to

achieve skin remediation or wound healing efficacy of the compositions of the present invention. In addition, although the carrier used in the pharmaceutical composition of the present invention varies slightly depending on various formulations, a carrier used according to a general formulation will be used.

The pharmaceutical composition of the present invention may be one having an improvement in skin condition or a wound treatment efficacy or activity. According to a more preferred embodiment, the improvement of the skin condition or wound treatment by the composition of the present invention is an effect of improving wrinkles, improving skin elasticity, preventing skin aging, improving skin moisturizing, treating acne, Is an effect of improving wrinkles, improving skin elasticity, preventing skin aging, removing wounds, and regenerating skin.

Hereinafter, the present invention will be described in more detail with reference to the following examples. However, the present invention is not limited by these examples.

(Preliminary Example) Culture and purification of EGF

Escherichia coli transformed with human EGF gene was cultured and then centrifuged at 9,000 rpm for 20 minutes to obtain E. coli supernatant. Ammonium sulfate was slowly added to the supernatant to make ammonium sulfate 30%, and the reaction was allowed to proceed at room temperature for 30 minutes, followed by centrifugation at 4,000 rpm for 20 minutes at 4 ° C. Ammonium sulfate was slowly added to the supernatant to make ammonium sulfate 60%. The reaction mixture was reacted at room temperature for 30 minutes and then centrifuged at 4,000 rpm for 30 minutes at 4,000 rpm for 30 minutes. The pellet was dissolved in 20 mM sodium phosphate (pH 5.8).

In order to remove insoluble matter, the supernatant was centrifuged at 12,000 rpm for 30 minutes at 4 ° C, and the supernatant was subjected to the first purification with 20 mM sodium phosphate (pH 5.8) using a Sephacryl HR200 column. The first purified EGF was subjected to a secondary purification using 20 mM sodium phosphate (pH 5.8) containing 0 to 0.5 M NaCl using a Bio-Scale Macro-Prep DEAE column. The purified EGF was dialyzed against 20 mM sodium phosphate (pH 5.8) and filtered through a 0.2 um filter.

(Example 1) Analysis of human EGF

20 mM sodium phosphate solution containing human EGF prepared by the present applicant (New Cell Life Co., Ltd.) was subjected to high performance liquid chromatography (HPLC) on a Waters XBridge C18 column to analyze its purity. The analytical conditions were as follows: Distilled water solution A containing 0.1% trifluoroacetic acid and acetonitrile solution B containing 0.1% trifluoroacetic acid were added at a rate of 1 ml / min from 0% B to 70% B Respectively.

Figure 1. The left figure shows that the peak of EGF was measured at 21.263 min and 12.131 min at RT (retention time), and the peak area corresponding to 11.263 min was 98.62% The purity of EGF used in this example was 98.62%. The peak at 11.263 min was reduced to 96.86%, 77.21%, and 38.60%, while the fine peak (1.38%) at 12.131 min was increased by heat treatment.

The results of SDS-PAGE analysis of the samples corresponding to purity of 96.66, 77.21% and 38.60% of 11.263 min are shown in P1, P2 and P3 of FIG. 1. The HPLC results show that the change in the purity of human EGF is due to the tertiary structure of human EGF And that there is no change in the primary structure.

(Example 2) Human skin fibroblast proliferation assay by the produced human EGF

The prepared 98.62% human EGF protein concentration was measured by a Bradford method on a spectrophotometer. At this time, BSA (Bovine Serum Albumin) at a concentration of 1 mg / ml was used as a standard sample of protein quantification. Human EGF standard samples were subjected to cell proliferation assays using EGF for research purchased from Peprotech Company (USA). The proliferative activity assay was performed in passage 3 human dermal fibroblast (HDF), a subculture of normal human skin cells to which the produced EGF was actually applied.

HDF cells were used in DMEM / Low glucose complete medium containing 10% heat-inactivated fetal bovine serum, 100 units / ml penicillin, and 100 mg / ml streptomycin. HDF cells were seeded at a density of 2×10^4 cells / well in a 24-well culture plate, and the HDF cells cultured for 16 hours were washed with PBS and then treated with EGF solution in DMEM / Low glucose medium containing 0.1% FBS And cultured for 72 hours. After incubation, 60 μl of CellTiter 96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, USA) was added and reacted for 4 hours. Cell viability was measured at 490 nm wavelength by taking 200 μl of the culture solution.

Figure 2 shows the growth of HDF (2 ng / ml) treated with 2 ng / ml of HDF (0.0 ng / ml) and EGF (NCL-EGF) The results showed that HDG cells treated with EGF at a concentration of 2 ng / ml had a high proliferation rate and thus had a relatively high cell concentration. As shown in the right-hand side of Figure 2, the HDF proliferative activity at 2 ng / ml of EGF for study was 1.93 at 490 nm wavelength, but the EGF produced at the same concentration 2.44, indicating that the HDF cell proliferating activity is very excellent.

In the same manner as in Example 1, the biological activity of 38.60% human EGF treated with 1 ng / ml of Example 1 was measured and the absorbance was 1.70 at a wavelength of 490 nm, which is a proliferation activity range similar to that of the manufacturer 0.25 ng / ml EGF Respectively. Thus, in the HPLC results of Example 1, the tertiary structure corresponding to the purity of RT at 11.263 min was the structure showing the biological activity of human EGF.

(Example 3) Glycerol activity assay as a heat stabilizer of human EGF

EGF prepared in Example 1 and stored in a freezer was diluted with 20 mM sodium phosphate (pH 5.8) and 20 mM sodium phosphate (pH 5.8) supplemented with 60% glycerol at a concentration of 100 ppm (100 ug / ml) ml in a 1.5 ml tube and stored at 4 ° C and 30 ° C. The results of HPLC analysis of the effect of glycerol on EGF thermal stability after storage at 4 ° C and 30 ° C for 7 days are shown in FIG.

FIG. 3 shows that when the 98.62% EGF used in the present example was stored at 4 ° C for 7 days, the purity and the purity when the glycerol was not added were 91.94% and 96.16%, respectively, decreased by 6.68% and 1.46% On the other hand, when the glycerol was added at 30 ℃ for 7 days, the purity was 22.07% when the glycerol was not added and the purity was 94.59% when the glycerol was 60%, which was decreased by 76.55% and 4.03%, respectively. In the present example, a composition containing 60% glycerol in 20 mM sodium phosphate (pH 5.8) solution stabilized 72.52% of EGF at 30 ° C than that without glycerol.

(Example 4) NaCl activity assay as a heat stabilizer of human EGF

The EGF prepared in Example 1 was diluted with 20 mM sodium phosphate (pH 5.8) and 20 mM sodium phosphate (pH 5.8) at a concentration of 100 ppm (100 μg / ml) ml tube and stored at 4 ° C. FIG. 4 shows the results of HPLC analysis of the effect of NaCl on EGF thermal stability at 24 hours intervals at 4 ° C.

PB refers to a sample diluted to 20 mM sodium phosphate, pH 5.8 with 100 ppm EGF, and 20 mM sodium phosphate, pH 5.8, with 100 ppm EGF and 20 mM NaCl added to Na-PB. 4, the structure was changed from 98.62% to 91.94% by 6.68% when stored at 4 ° C for 144 hours (6 days) while 100 ppm EGF was

diluted with PB, whereas in PB containing 20 mM NaCl, the structure It was 1.73% modified from 98.62% to 96.89%. Thus, in this example, 20 mM NaCl added when storing EGF under the same conditions served as a stabilizer to stabilize the EGF structure about 3.86-fold.

(Example 5) Analysis of thermal stability of human natural EGF at 30 占 폆

The prepared human EGF was diluted with 20 mM sodium phosphate, pH 5.8, and 20 mM sodium phosphate, pH 5.8, to a concentration of 100 ppm (100 ug / ml) And stored at 4 ° C and 30 ° C. The thermal stability of human EGF was analyzed by HPLC at intervals of 24 hours.

FIG. 5 shows the percentage of the native structure of human EGF in terms of temperature and time, and FIG. 6 shows HPLC chromatograms of structural changes of human EGF at 4 ° C and 30 ° C temperature and time . Even though the human EGF stabilizer 20 mM NaCl was contained in the aqueous solution, it was found that after 24 hours at 30 ° C, the natural EGF was abruptly changed from 98.62% to 77.21% and after 48 hours, 41.16% there was. After 48 hours at 30 ° C, the structure of human natural EGF was found to be progressing more slowly than before 48 hours. In this example, the sodium chloride composition was found to stabilize the EGF tertiary structure at lower temperatures at relatively high temperatures such as 30 < 0 > C.

(Example 6) Activity test of glycerol as a structural stabilizer of human natural EGF according to temperature and time conditions

A 98.62% human wild-type EGF prepared in order to analyze the activity of glycerol as a heat stabilizer for the tertiary structure of the wild type EGF was prepared by adding glycerol at a weight ratio (w / v) of 0, 10, 30, 50 and 60% Diluted to 100 ppm (100 ug / ml) in 20 mM NaCl / 20 mM sodium phosphate, pH 5.8 and stored at 4 ° C and 30 ° C in 1 ml aliquots in 1.5 ml tubes. The results are shown in Table 1.

글리세롤 농도 / 온도 및 시간		0%	10%	30%	50%	60%
4 ℃	24 hr	98.65	98.45	98.75	98.75	98.44
	48 hr	98.60	98.80	98.24	98.46	98.36
	72 hr	98.01	96.70	98.35	98.71	98.38
	96 hr	97.67	93.51	98.46	98.59	98.52
	120 hr	97.21	64.72	98.05	98.17	98.30
	144 hr	96.89	45.98	98.00	98.35	98.47
30 ℃	24 hr	80.77	90.61	97.67	98.55	98.67
	48 hr	47.88	48.11	98.42	97.95	98.65
	72 hr	43.08	37.52	97.92	98.42	98.64
	96 hr	35.32	31.59	96.61	98.21	98.68
	120 hr	34.92	27.21	96.19	98.42	98.62
	144 hr	32.99	24.55	95.97	98.53	98.59

온도 및 시간 \ 글리세롤 농도		0%	10%	30%	50%	60%
4 ℃	24 hr	98.65	98.45	98.75	98.75	98.44
	48 hr	98.60	98.80	98.24	98.46	98.36
	72 hr	98.01	96.70	98.35	98.71	98.38
	96 hr	97.67	93.51	98.46	98.59	98.52
	120 hr	97.21	64.72	98.05	98.17	98.30
	144 hr	96.89	45.98	98.00	98.35	98.47
30 ℃	24 hr	80.77	90.61	97.67	98.55	98.67
	48 hr	47.88	48.11	98.42	97.95	98.65
	72 hr	43.08	37.52	97.92	98.42	98.64
	96 hr	35.32	31.59	96.61	98.21	98.68
	120 hr	34.92	27.21	96.19	98.42	98.62
	144 hr	32.99	24.55	95.97	98.53	98.59

The results of auto-scale analysis of the samples containing glycerol 30% or more showed an error of about 1%. The natural EGF purity of the sample stored at 4 ° C for 144 hours was more than 2 times higher than that of the non-glycerol-containing sample (96.89%) and the tertiary structure of the native EGF was more than twice as high as that of the sample containing 10% glycerol (45.98% . Similarly, the natural EGF purity of the sample stored at 30 ° C for 144 hours was about 8% higher than that of the non-glycerol-containing sample (32.99%) in a sample containing 10% glycerol (24.55% % More.

The peculiarity of the invention as identified in this example is that the purity of the native EGF of the sample stored at 30 ° C. for 144 hours was reduced by 65.63% in the glycerol-free sample (32.99%) while in the sample containing 30% glycerol 2.65%, and no change in tertiary structure was observed in samples containing more than 50% glycerol. As a characteristic result of the above Table 1, the thermal stability of human natural EGF stored at 4 ° C. and 30 ° C. for 144 hours was compared and analyzed by glycerol concentration, and it is shown in FIG. 7 and FIG.

The graph of FIG. 7 shows the result of comparative analysis of HPLC results after storage for 144 hours at each temperature condition. The HPLC chromatogram of FIG. 8 shows the EGF tertiary structure (red line portion). ≪ / RTI > From the results of this example, it was found that a composition containing 30% or more of glycerol in 20 mM sodium phosphate (pH 5.8) / 20 mM sodium chloride stabilized human EGF tertiary structure by 98% or more for 144 hours at 30 ° C, It appears that the included composition acts as a thermal stabilizer to preserve the complete human EGF tertiary structure within the error range.