WHAT IS CLAIMED IS:

1. A method for preparing a complex of physiologically active polypeptide - non-peptidyl polymer - immunoglobulin constant region, comprising the steps of:

(1) reacting a non-peptidyl polymer with one of a physiologically active polypeptide or an immunoglobulin constant region; and

(2) reacting the reaction mixture of step (1) with the other of the physiologically active polypeptide or the immunoglobulin constant region in the presence of a salt at a concentration of 0.3 to 3M.

2. The method according to claim 1, wherein the non-peptidyl polymer has each independently a functional group selected from the group consisting of an aldehyde derivative, a maleimide derivative and a succinimide derivative at both ends thereof.

3. The method according to claim 2, wherein the non-peptidyl polymer is linked to the physiologically active polypeptide and the immunoglobulin constant region via the functional groups at both ends thereof to form a covalent bond.

4. The method according to claim 1, further comprising the step of separating a conjugate of physiologically active polypeptide - non-peptidyl polymer or a conjugate of immunoglobulin constant region - non-peptidyl polymer from the reaction mixture after step (1).

5. The method according to claim 1, wherein the salt is selected from the group consisting of sodium chloride, sodium acetate, sodium sulfate, sodium phosphate, sodium carbonate, sodium cyanide, sodium citrate, sodium nitrate, potassium chloride, potassium acetate, potassium sulfate, potassium phosphate, potassium carbonate, potassium cyanide, potassium citrate, potassium nitrate, magnesium chloride, magnesium acetate, magnesium sulfate, magnesium phosphate, magnesium carbonate, magnesium cyanide, magnesium citrate, magnesium nitrate, ammonium chloride, ammonium acetate, ammonium sulfate, ammonium phosphate, ammonium carbonate, ammonium cyanide, ammonium citrate, ammonium nitrate, calcium chloride, calcium acetate, calcium sulfate, calcium phosphate, calcium carbonate, calcium cyanide, calcium citrate, and calcium nitrate.

6. The method according to claim 5, wherein the salt is selected from the group consisting of sodium chloride,

sodium acetate, sodium sulfate, sodium phosphate and potassium chloride.

~~7. The method according to claim 1, wherein the salt is added at a final concentration of 0.1 to 3.0 M.~~

~~8~~7. The method according to claim ~~7~~1, wherein the salt is added at a final concentration of 0.3 to 2.5 M.

~~9~~8. The method according to claim 6, wherein if the salt is sodium chloride, it is added at a final concentration of less than 3.0 M, if the salt is sodium acetate, it is added at a final concentration of less than 2.5 M, if the salt is sodium sulfate, it is added at a final concentration of less than 0.7 M, if the salt is sodium phosphate, it is added at a final concentration of less than 0.8 M, or if the salt is potassium chloride, it is added at a final concentration of 1.0 M or less.

~~10~~9. The method according to claim 1, wherein the reaction time of step (2) is 4 to 18 hours.

~~11~~10. The method according to claim 1, wherein the reaction temperature of step (2) is 0 to 25°C.

~~12~~11. The method according to claim 2, wherein if the non-peptidyl polymer has one or more aldehyde derivative as functional groups, the reaction mixture further comprises a reducing agent at a final concentration of 1 to 100 mM.

~~13~~12. The method according to claim 1, wherein step (1) is carried out at pH 5.0 to 6.5 and step (2) is carried out at pH 6.0 to 8.5.

~~14~~13. The method according to claim 1, wherein the non-peptidyl polymer reacts with the physiologically active polypeptide in step (1), and the reaction mixture of step (1) reacts with the immunoglobulin constant region in step (2).

~~15~~14. The method according to claim ~~14~~13, wherein the physiologically active polypeptide and the non-peptidyl polymer react with each other at a molar ratio of 1:1 to 1:20 in step (1), and the product of step (1) and the immunoglobulin constant region react with each other at a molar ratio of 1:0.5 to 1:10 in step (2).

~~16~~15. The method according to claim ~~15~~14, wherein step (2) is carried out in the presence of sodium chloride added at a final concentration of less than 3.0 M, sodium acetate added at a final concentration of less than 2.5 M, sodium

sulfate added at a final concentration of less than 0.7 M, sodium phosphate added at a final concentration of less than 0.8 M, or potassium chloride added at a final concentration of 1.0 M or less.

~~17~~16. The method according to claim 1, wherein the functional groups of the non-peptidyl polymer are linked to an amine group which is present at an N-terminus or on a side chain of Lys residue of the physiologically active polypeptide and the immunoglobulin constant region.

~~18~~17. The method according to claim 1, wherein the non-peptidyl polymer is selected from the group consisting of polyethylene glycols, polypropylene glycols, copolymers of ethylene glycol and propylene glycol, polyoxyethylated polyols, polyvinyl alcohols, polysaccharides, dextrans, polyvinyl ethyl ethers, polylactic acid (PLA), polylactic-glycolic acid (PLGA), lipid polymers, chitins, hyaluronic acid, and the combination thereof.

~~19~~18. The method according to claim 1, wherein the non-peptidyl polymer is polyethylene glycol.

~~20~~19. The method according to claim 1, wherein the non-peptidyl polymer has a molecular weight ranging from 1 to 100 kDa.

~~21~~20. The method according to claim 1, wherein the immunoglobulin constant region is aglycosylated.

~~22~~21. The method according to claim 1, wherein the immunoglobulin constant region consists of one to four domains selected from the group consisting of CH1, CH2, CH3 and CH4 domains.

~~23~~22. The method according to claim 1, wherein the immunoglobulin constant region further comprises a hinge region.

~~24~~23. The method according to claim 1, wherein the immunoglobulin constant region is selected from the group consisting of constant regions derived from IgG, IgA, IgD, IgE, IgM, combinations thereof, and hybrids thereof.

~~25~~24. The method according to claim 1, wherein the immunoglobulin constant region is selected from the group consisting of constant regions of IgG1, IgG2, IgG3, IgG4, combinations thereof, and hybrids thereof.

~~26~~25. The method according to claim ~~25~~24, wherein the immunoglobulin constant region is an IgG4 Fc region.

~~27~~26. The method according to claim ~~26~~25, wherein the immunoglobulin constant region is an aglycosylated human IgG4 Fc region.

~~28~~27. The method according to claim 1, wherein the physiologically active polypeptide is selected from the group consisting of human growth hormone, growth hormone releasing hormones, growth hormone releasing peptides, interferon, interferon receptors, colony-stimulating factors, glucagon-like peptides (GLP-1, etc.), oxyntomodulin, G protein-coupled receptors, interleukins, interleukin receptors, enzymes, interleukin-binding proteins, cytokine-binding proteins, macrophage activating factors, macrophage peptides, B-cell factors, T-cell factors, Protein A, allergy inhibitors, cell necrosis glycoproteins, immunotoxins, lymphotoxins, tumor necrosis factor, tumor suppressors, transforming growth factor, alpha-1 anti-trypsin, albumin, α-lactalbumin, apolipoprotein-E, erythropoietin, glycosylated erythropoietin, angiopoeitins, hemoglobin, thrombin, thrombin receptor activating peptides, thrombomodulin, blood factor VII, VIIa, VIII, IX and XIII,

plasminogen activators, fibrin-binding peptides, urokinase, streptokinase, hirudin, Protein C, C-reactive protein, renin inhibitor, collagenase inhibitor, superoxide dismutase, leptin, platelet-derived growth factor, epithelial growth factor, epidermal growth factor, angiostatin, angiotensin, bone growth factor, bone stimulating protein, calcitonin, insulin, atriopeptin, cartilage inducing factor, elcatonin, connective tissue activating factor, tissue factor pathway inhibitor, follicle stimulating hormone, luteinizing hormone, luteinizing hormone releasing hormone, nerve growth factors, parathyroid hormone, relaxin, secretin, somatomedin, insulin-like growth factor, adrenocortical hormone, glucagon, cholecystokinin, pancreatic polypeptide, gastrin releasing peptide, corticotropin releasing factor, thyroid stimulating hormone, autotaxin, lactoferrin, myostatin, cell surface antigens, virus-derived vaccine antigens, monoclonal antibodies, polyclonal antibodies, and antibody fragments.

~~29~~28. The method according to claim ~~28~~27, wherein the physiologically active polypeptide is insulin.

~~30~~29. The method according to claim 1, wherein in step (1), the physiologically active polypeptide and the non-peptidyl polymer react with each other at a molar ratio of 1:1 to 1:20 under the pH condition of 5.0 to 6.5, and in

step (2), the reaction mixture of step (1) and the immunoglobulin constant region react with each other at a molar ratio of 1:0.5 to 1:10 under the pH condition of 6.0 to 8.5 in the presence of the salt, and if the salt is sodium chloride, it may be added at a final concentration of less than 3.0 M, if the salt is sodium acetate, it may be added at a final concentration of less than 2.5 M, if the salt is sodium sulfate, it may be added at a final concentration of less than 0.7 M, if the salt is sodium phosphate, it may be added at a final concentration of less than 0.8 M, or if the salt is potassium chloride, it may be added at a final concentration of 1.0 M or less.