

of GL-2045 that mimic the enhanced efficacy of the multimeric fractions of IVIG. Standard cell culture conditions, however, produce varying levels of cell viability, degrees of multimerized proteins, and protein titers. Therefore, there is a need in the art for methods of manufacturing GL-2045 that results in a defined multimer pattern, and particularly one that results in an increased percentage of higher-order multimers while optimizing cell viability and protein titer.

SUMMARY

[0009] The present disclosure provides for all three of improved cell viability, improved high protein titer, and a surprising and substantial increase in the percentage of higher-order multimers relative to standard manufacturing techniques. This optimized manufacturing method therefore, provides for optimally manufactured GL-2045 compositions with enhanced efficacy for treating inflammatory diseases as compared with non-optimally manufactured GL-2045 compositions. Optimized manufacturing of GL-2045 includes optimized upstream manufacturing methods and, in some examples optimized downstream methods. Optimized upstream manufacturing methods a) generate high protein titers, b) maintain high cell viability to minimize cell debris, and c) retain both the highly ordered multimers of the homodimer that are essential for the functioning of GL-2045 and, if desired, the homodimer. Optimized downstream manufacturing methods include various purification techniques that are employed specifically to maintain a selected multimer profile of GL-2045. Thus, in some examples, provided herein are GL-2045 compositions with a defined multimer profile.

[0010] In some examples, a method for producing GL-2045 is provided comprising culturing Chinese Hamster Ovary (CHO) cells that have been stably transfected with an expression vector encoding GL-2045 at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until the CHO cells reach a cell density of about 5 to about 30 million cells/mL; shifting the growth temperature from $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ to $32.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$; and harvesting GL-2045 from the culture media. In some examples, the cells are grown to a density of about 10 to about 25 million cells/mL prior to the shifting growth temperature. In some examples, the cells are grown to a density of about 10 to about 15 million cells/mL prior to the shifting growth temperature. In some examples, the cells are grown to a density of about 15 to about 20 million cells/mL prior to the shifting growth temperature. In some examples, a dual temperature shift is employed with a shift from $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ to $34^{\circ}\text{C} \pm 1^{\circ}\text{C}$ on about day 3 with a second temperature shift from $34^{\circ}\text{C} \pm 1^{\circ}\text{C}$ to $31^{\circ}\text{C} \pm 1^{\circ}\text{C}$ on about day 7 of bioreactor culture.

[0011] In some examples, the CHO cells are cultured in in ActiCHO P base culture media. In some examples, the CHO cells are fed during culture with ActiCHO feed A and ActiCHO feed B. In some examples, the CHO cells are fed every other day. In some examples, the expression vector encoding GL-2045 comprises the leader peptide of SEQ ID NO: 1. In some examples, the expression vector encoding GL-2045 further comprises a piggyBac transposase recognition sequence and is transfected with a vector encoding a piggyBac transposase. In some examples, the expression vector encoding GL-2045 results in fewer than 20 genomic insertions.

[0011a] In one aspect, the present disclosure refers to a method for producing a composition comprising a homodimer and/or a multimer of the homodimer, wherein the homodimer comprises two monomers each comprising amino acids 21-264 of SEQ ID NO:4, the method comprising:

- (a) culturing Chinese Hamster Ovary (CHO) cells that have been stably transfected with an expression vector comprising a nucleic acid sequence encoding SEQ ID NO: 4 at a growth temperature of $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. for 5 days;
- (b) shifting the growth temperature from $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. to $32.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$.; and
- (c) harvesting the homodimer and/or the multimer of the homodimer from the culture media.

[0012] In some examples, a recombinantly produced GL-2045 made by the methods described herein is provided. In some examples, an expression vector is provided encoding GL-2045 comprising a GL-2045 expression cassette, wherein the GL-2045 expression cassette is flanked by piggyBac minimal inverted repeat elements.

[0013] In some examples, a method for producing GL-2045 is provided comprising transfecting CHO cells with an expression vector described herein, culturing the CHO cells in a bioreactor with ActiCHO P media at a growth temperature of $37^{\circ}\text{C} \pm 1^{\circ}$, feeding the cultures of CHO cells with Acti CHO Feed A and Acti CHO Feed B daily at a growth temperature of $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until the cultures reach a cell density of about 10 million to about 15 million cells/mL, shifting the growth temperature from $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ to $32.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$, and harvesting GL-2045 from the culture media, wherein the methods result in a cell viability of $>80\%$ at Day 21, and a final protein titer of $> 9,000\text{ mg/mL}$ of which $>70\%$ of GL-2045 is present as a multimer, wherein $>30\%$ of the multimers are higher order multimers GL-2045. In some examples, the cell viability exceeds 95% at day 18 of culture. In some examples, the percent of multimers exceeds 80% .

[0013a] In one aspect, the present disclosure refers to a method for producing a composition comprising a homodimer and/or a multimer of the homodimer, wherein the homodimer comprises two monomers each comprising amino acids 21-264 of SEQ ID NO:4, the method comprising:

- (a) transfecting Chinese Hamster Ovary (CHO) cells with an expression vector comprising a nucleic acid encoding SEQ ID NO: 4;
- (b) culturing the CHO cells from (a) in a bioreactor with ActiCHO P media at a growth temperature of 37° C.±1°;
- (c) feeding the cultures of (b) with Acti CHO Feed A and Acti CHO Feed B daily at a growth temperature of 37° C.±1° C. for 5 days;
- (d) shifting the growth temperature from 37° C.±1° C. to 32.5° C.±1° C.; and
- (e) harvesting the homodimer and/or multimer of the homodimer from the culture media, wherein >70% of the composition is present as the multimer of the homodimer, and wherein >30% of the multimers are higher order multimers.

[0013b] In one aspect, the present disclosure refers to a method for producing a composition comprising a homodimer and/or a multimer of the homodimer, wherein the homodimer comprises two monomers each comprising amino acids 21-264 of SEQ ID NO:4, the method comprising:

- (a) culturing Chinese Hamster Ovary (CHO) cells, that have been stably transfected with an expression vector comprising a nucleic acid sequence encoding SEQ ID NO: 4, at a growth temperature of 37° C.±1° C. until the CHO cells reach a cell density of about 5 to about 30 million cells/mL;
- (b) shifting the growth temperature from 37° C.±1° C. to 32.5° C.±1° C.; and
- (c) harvesting the homodimer and/or the multimer of the homodimer from the culture media.

[0014] In some examples, a method of purifying GL-2045 produced by the methods described herein is provided comprising purifying GL-2045 from the culture supernatant by affinity chromatography and polishing GL-2045 by one or more of cation exchange chromatography, anion exchange chromatography, and hydrophobic interaction chromatography.

[0015] In some examples, depth filtration is employed prior to affinity chromatography. In some examples, the depth filter is the X0HC (Millipore). In some examples, the depth filtration unit removes a high percentage of DNA from supernatant. In some examples that depth filtration unit is Emphaze™ AEX Hybrid Purifier (3M).

[0016] In some examples, the affinity chromatography uses a protein A column. In some examples, the protein A column comprises an NaOH-resistant resin. In some examples, the protein A resin is a MabSelect SuRe resin. In some examples, purification by affinity chromatography comprises utilizing one of three different wash buffers to optimize purification conditions. In some examples, purification by affinity chromatography comprises eluting GL-2045 from the affinity chromatography column. In some examples, eluting GL-2045 comprises elution with a pH gradient. In some examples, eluting GL-2045 comprises elution without a pH gradient. In one example, elution is performed using a glycine buffer. In another example, elution is performed using an acetic acid buffer. In some examples, the affinity chromatography column is regenerated to remove bound GL-2045. In some examples, the affinity chromatography column is regenerated more frequently than suggested by the manufacturer. In some examples, the affinity chromatography column is regenerated prior to each purification cycle. In some examples, the affinity chromatography column is regenerated with a 0.5 M NaOH buffer.

[0017] In some examples, polishing GL-2045 comprises anion exchange flow through chromatography. In some examples, anion exchange flow through chromatography comprises using a Q Sepharose Fast Flow column. In some examples, polishing GL-2045 comprises cation exchange chromatography. In some examples, cation exchange chromatography comprises using a POROS XS column. In some examples, cation exchange chromatography comprises using a sodium acetate elution buffer. In some examples, the elution buffer further comprises 36.5-39.0% of a 1 M NaCl buffer. In one example, the elution method is step elution and in another example the elution is gradient elution. In some examples, polishing GL-2045 comprises hydrophobic interaction chromatography. In some examples, hydrophobic interaction chromatography comprises using a Butyl FF resin. In some examples, hydrophobic interaction chromatography comprises using a Phenyl HP resin. In some examples, hydrophobic interaction chromatography (“HIC”) comprises using a Phenyl Sepharose 6 Fast Flow High Sub resin. In one example, the HIC method is in flow through mode and in another example the HIC method is in binding mode. In some examples, the HIC resin results in isolation of the GL-2045 homodimer. In some examples, hydrophobic interaction chromatography comprises using an Octyl FF resin. In some examples, the column results in the removal of un-ordered aggregates of GL-2045.

[0018] In some examples, a method for purifying GL-2045 is provided comprising purifying GL-2045 from the culture supernatant by protein A affinity chromatography, wherein the protein A column uses an alkaline-resistant medium such as the MabSelect SuRe medium, wherein the purification is performed with at least two wash cycles, and wherein clean in place (CIP) procedures are performed after each purification run with a high NaOH regeneration step such as 0.5 M NaOH buffer.

[0019] In some examples, a method for purifying GL-2045 is provided comprising polishing GL-2045 by cation exchange chromatography, wherein the cation exchange column contains a high-capacity, high-resolution resin such as POROS XS and wherein the elution buffer is a sodium acetate buffer comprised of 36.5-39.0% of a 1 M NaCl buffer. In some examples, the method for purifying GL-2045 further comprises polishing GL-2045 by anion exchange chromatography, wherein the anion exchange column contains a strong anion exchange medium that has high chemical stability, allowed clean-in-place and sanitation protocols, such as the Q Sepharose Fast Flow medium. In some examples, the method for purifying GL-2045 further comprises polishing GL-2045 by hydrophobic interaction chromatography, wherein the hydrophobic interaction medium is a Butyl FF, a Phenyl HP, or an Octyl FF resin and is selected to isolate or remove a particular fraction of GL-2045 in addition to polishing. In some examples, the method for purifying and/or polishing GL-2045 results in a final protein titer of GL-2045 > 4 g/L after all filtration and chromatography steps (i.e. the final Drug Substance). In some examples, the final protein composition of GL-2045 comprises >70% multimers. In some examples, >28% of the multimers are higher order multimers as analyzed by analytical SEC-HPLC.

[0019a] In one aspect, the present disclosure refers to a method of purifying the homodimer and/or multimer of the homodimer produced by the method of claim 1 comprising:

- (a) purifying the homodimer and/or multimer of the homodimer from the culture supernatant by affinity chromatography; and
- (b) polishing the homodimer and/or multimer of the homodimer by one or more of cation exchange chromatography, anion exchange chromatography, and hydrophobic interaction chromatography.

[0019b] In one aspect, the present disclosure refers to a method for purifying and polishing a composition comprising a homodimer and/or a multimer of the homodimer, wherein the homodimer comprises two monomers each comprising amino acids 21-264 of SEQ ID NO:4 comprising:

(a) purifying the homodimer and/or multimer of the homodimer from a culture supernatant by protein A affinity chromatography, wherein the protein A chromatography uses an alkaline-resistant medium such as the MabSelect SuRe medium, wherein purifying is performed with at least two wash cycles;

(b) polishing the homodimer and/or multimer of the homodimer by cation exchange chromatography, wherein the cation exchange chromatography uses a high-capacity, high-resolution resin such as POROS XS, and wherein the elution buffer is a sodium acetate buffer comprised of 36.5-38.5% of a 1 M NaCl buffer;

(c) polishing the homodimer and/or multimer of the homodimer by anion exchange chromatography, wherein the anion exchange chromatography uses an anion exchange medium such as the Q Sepharose Fast Flow medium; and

(d) polishing the homodimer and/or multimer of the homodimer by hydrophobic interaction chromatography (HIC), wherein the HIC medium is Phenyl Sepharose 6 Fast Flow High Sub Resin and is selected to isolate or remove a particular fraction of the multimer of the homodimer in addition to polishing.

[0020] In some examples, a purified GL-2045 made by the methods described herein is provided. In some examples, the purified GL-2045 made by the methods described herein has a defined multimer pattern that minimizes the percentage of homodimers and/or dimers of the homodimers, or otherwise balances the percentage of homodimers, lower order multimers, higher order multimers, and highest order multimers. In some examples, a method of treating or preventing an inflammatory, autoimmune or infections disease or disorder in a subject in need thereof with the recombinantly produced, purified GL-2045 described herein is provided. In some examples, the disease or disorder is selected from idiopathic thrombocytopenic purpura, chronic inflammatory polyneuropathy, multifocal motor neuropathy, myasthenia gravis, organ

transplantation, and rheumatoid arthritis. In some examples, the GL-2045 is administered intravenously, subcutaneously, orally, intraperitoneally, sublingually, buccally, transdermally, via subdermal implant or intramuscularly.

[0021] In some examples, a recombinantly produced GL-2045 composition is provided, wherein the homodimer fraction of the GL-2045 composition comprises less than about 20% of the total composition. In some examples, the homodimer fraction comprises 12-19% of the total composition. In other examples, the homodimer fraction comprises 14-19% of the total composition. In some examples, the homodimer fraction comprises 15.5-17.5% of the total composition. In another examples, the homodimer fraction comprises about 16.2% of the total composition.

[0022] In some examples, a recombinantly produced GL-2045 composition is provided wherein the dimer of the homodimer fraction of the GL-2045 composition comprises about 7% to about 12% of the total composition. In some examples, the dimer of the homodimer fraction comprises about 9% to about 11% of the total composition. In other examples, the dimer of the homodimer fraction comprises about 10% of the total composition.

[0023] In some examples, a recombinantly produced GL-2045 composition is provided, wherein the trimer of the homodimer fraction of the GL-2045 composition comprises about 5.5% to about 11% of the total composition. In some examples, the trimer of the homodimer fraction comprises about 6.5% to about 8% of the total composition. In other examples, the timer of the homodimer fraction comprises about 7% of the total composition.

[0024] In some examples, a recombinantly produced GL-2045 composition is provided, wherein the tetramer of the homodimer fraction of the GL-2045 composition comprises about 10% to about 16% of the total composition. In some examples, the tetramer of the homodimer fraction comprises about 13% to about 15% of the total composition. In other examples, the tetramer of the homodimer fraction comprises about 14% of the total composition.

[0025] In some examples, a recombinantly produced GL-2045 composition is provided wherein the pentamer of the homodimer fraction of the GL-2045 composition comprises about 6% to about 9% of the total composition. In some examples, the pentamer of the homodimer fraction comprises about 7% to about 8% of the total composition. In other

examples, the pentamer of the homodimer fraction comprises about 7% of the total composition.

[0026] In some examples, a recombinantly produced GL-2045 composition is provided, wherein the hexamer of the homodimer fraction of the GL-2045 composition comprises about 10% to about 14% of the total composition. In some examples, the hexamer of the homodimer fraction comprises about 12% to about 13% of the total composition. In other examples, the hexamer of the homodimer fraction comprises about 12.7% of the total composition.

[0027] In some examples, a recombinantly produced GL-2045 composition is provided wherein the highest order multimers (*i.e.*, those in the 7-mer of the homodimer and above fractions) comprise at least about 28% of the total composition. In some examples, the highest order multimers comprise no more than 35% of the total composition. In some examples, the highest order multimer fractions comprise from about 30% to about 34% of the total composition. In other examples, the highest order multimer fractions comprise about 31.4% of the total composition.

[0028] In some examples, a recombinantly produced GL-2045 composition is provided wherein

- (a) the homodimeric fraction comprises less than about 20% of the total composition;
- (b) the highest order multimer fractions comprise at least about 28% of the total composition;
- (c) the dimer of the homodimer fraction comprises from about 7% to about 12.5% of the total composition;
- (d) the trimer of the homodimer fraction comprises from about 5.5% to about 11% of the total composition;
- (e) the tetramer of the homodimer fraction comprises from about 10% to about 16% of the total composition;
- (f) the pentamer of the homodimer fraction comprises from about 6% to about 10% of the total composition;
- (g) the hexamer of the homodimer fraction comprises from about 10% to about 14% of the total fraction;

(h) the dimer of the homodimer through hexamer of the homodimer fraction comprises from about 40% to about 60% of the total composition;

(i) the trimer of the homodimer through the hexamer of the homodimer fractions comprise from about 32% to about 50% of the total composition;

(j) the tetramer of the homodimer through the hexamer of the homodimer fraction comprise from about 26% to about 39% of the total composition;

(k) the pentamer of the homodimer through the hexamer of the homodimer fraction comprise from about 18% to about 23% of the total composition; or

(l) any combination of (a)-(k).

[0029] In some examples, a recombinantly produced GL-2045 composition is provided, wherein approximately 80% of the total composition comprises higher order multimers, meaning the dimer of the homodimer and above (*i.e.*, band 2 and above). In some examples, approximately 60-80% of the total recombinantly produced GL-2045 composition comprises the trimer of the homodimer and above (*i.e.*, band 3 and above). In some examples, about 54-72% of the total of the recombinantly produced GL-2045 composition comprises the tetramer and above (*i.e.*, band 4 and above). In some examples, a GL-2045 is provided wherein approximately 44-57% of the total composition comprises the pentamer and above (*i.e.*, band 5 and above). In some examples, about 38-51% of the total of the recombinantly produced GL-2045 composition comprises the hexamer and above (*i.e.*, band 6 and above).

[0030] In some examples, a recombinantly produced GL-2045 is provided wherein bands 2-6 of the composition (*i.e.*, the dimer of the homodimer through the hexamer of the homodimer) comprise about 39-61% of the composition. In some examples, a recombinantly produced GL-2045 is provided, wherein bands 3-6 of the composition (*i.e.*, the trimer of the homodimer through the hexamer of the homodimer) comprises about 32-50% of the composition. In some examples, a recombinantly produced GL-2045 is provided wherein bands 4-6 of the composition (*i.e.*, the tetramer of the homodimer through the hexamer of the homodimer) comprises about 26-39% of the composition. In some examples, a recombinantly produced GL-2045 is provided wherein bands 5-6 of the composition (*i.e.*, the pentamer of the homodimer through the hexamer of the homodimer) comprises about 16-23% of the composition.

BRIEF DESCRIPTION OF THE DRAWINGS

CLAIMS

1. A method for producing a composition comprising a homodimer and/or a multimer of the homodimer, wherein the homodimer comprises two monomers each comprising amino acids 21-264 of SEQ ID NO:4, the method comprising:

(a) culturing Chinese Hamster Ovary (CHO) cells that have been stably transfected with an expression vector comprising a nucleic acid sequence encoding SEQ ID NO: 4 at a growth temperature of 37° C.±1° C. for 5 days;

(b) shifting the growth temperature from 37° C.±1° C. to 32.5° C.±1° C.; and

(c) harvesting the homodimer and/or the multimer of the homodimer from the culture media.

2. The method of claim 1, wherein the cells are grown to a density of about 10 to about 25 million cells/mL, to a density of about 10 to about 15 million cells/mL, or to a density of about 15 to about 20 million cells/mL prior to shifting the growth temperature.

3. The method of claim 1, wherein the culturing is done in ActiCHO P base culture media comprising glutamine and wherein the CHO cells are fed during culture with ActiCHO feed A and ActiCHO feed B.

4. The method of claim 1, wherein the expression vector comprising the nucleic acid sequence encoding SEQ ID NO: 4 comprises a nucleic acid sequence encoding the leader peptide of SEQ ID NO: 1.

5. The method of claim 1, wherein the expression vector comprising a nucleic acid sequence encoding SEQ ID NO: 4 further comprises a piggyBac transposase recognition sequence and is transfected with a vector encoding a piggyBac transposase.

6. The method of claim 1, wherein the expression vector results in less than 20 genomic insertions.

7. A method for producing a composition comprising a homodimer and/or a multimer of the homodimer, wherein the homodimer comprises two monomers each comprising amino acids 21-264 of SEQ ID NO:4, the method comprising:

(a) transfecting Chinese Hamster Ovary (CHO) cells with an expression vector comprising a nucleic acid encoding SEQ ID NO: 4;

(b) culturing the CHO cells from (a) in a bioreactor with ActiCHO P media at a growth temperature of 37° C.±1° C.;

(c) feeding the cultures of (b) with Acti CHO Feed A and Acti CHO Feed B daily at a growth temperature of 37° C.±1° C. for 5 days;

(d) shifting the growth temperature from 37° C.±1° C. to 32.5° C.±1° C.; and

(e) harvesting the homodimer and/or multimer of the homodimer from the culture media, wherein >70% of the composition is present as the multimer of the homodimer, and wherein >30% of the multimers are higher order multimers.

8. The method of claim 7, wherein cell viability exceeds 85% at day 18 of culture.

9. The method of claim 7, wherein greater than 80% of the composition comprises the multimer of the homodimer.

10. A method of purifying the homodimer and/or multimer of the homodimer produced by the method of claim 1 comprising:

(a) purifying the homodimer and/or multimer of the homodimer from the culture supernatant by affinity chromatography; and

(b) polishing the homodimer and/or multimer of the homodimer by one or more of cation exchange chromatography, anion exchange chromatography, and hydrophobic interaction chromatography.

11. The method of claim 10, wherein the affinity chromatography uses a Mab Select SuRe protein A column.

12. The method of claim 10, wherein purification by affinity chromatography comprises

eluting the homodimer and/or multimer of the homodimer from the affinity chromatography column using an elution buffer comprising sodium acetate and NaCl.

13. The method of claim 11, wherein the protein A column is regenerated with an NaOH buffer of at least 0.5 M.

14. The method of claim 10, wherein polishing the homodimer and/or multimer of the homodimer comprises anion exchange flow through chromatography.

15. The method of claim 14, wherein anion exchange flow through chromatography comprises using a Q Sepharose Fast Flow column.

16. The method of claim 10, wherein polishing the homodimer and/or multimer of the homodimer comprises cation exchange chromatography.

17. The method of claim 16, wherein cation exchange chromatography comprises using a POROS XS column.

18. The method of claim 16, wherein cation exchange chromatography comprises using a sodium acetate elution buffer.

19. The method of claim 18, wherein the elution buffer further comprises 36.5-38.5% of a 1 M NaCl buffer.

20. The method of claim 10, wherein polishing the homodimer and/or multimer of the homodimer comprises hydrophobic interaction chromatography.

21. The method of claim 20, wherein hydrophobic interaction chromatography comprises using a Phenyl Sepharose 6 Fast Flow High Sub Resin.

22. A method for purifying and polishing a composition comprising a homodimer and/or a

multimer of the homodimer, wherein the homodimer comprises two monomers each comprising amino acids 21-264 of SEQ ID NO:4 comprising:

(a) purifying the homodimer and/or multimer of the homodimer from a culture supernatant by protein A affinity chromatography, wherein the protein A chromatography uses an alkaline-resistant medium such as the MabSelect SuRe medium, wherein purifying is performed with at least two wash cycles;

(b) polishing the homodimer and/or multimer of the homodimer by cation exchange chromatography, wherein the cation exchange chromatography uses a high-capacity, high-resolution resin such as POROS XS, and wherein the elution buffer is a sodium acetate buffer comprised of 36.5-38.5% of a 1 M NaCl buffer;

(c) polishing the homodimer and/or multimer of the homodimer by anion exchange chromatography, wherein the anion exchange chromatography uses an anion exchange medium such as the Q Sepharose Fast Flow medium; and

(d) polishing the homodimer and/or multimer of the homodimer by hydrophobic interaction chromatography (HIC), wherein the HIC medium is Phenyl Sepharose 6 Fast Flow High Sub Resin and is selected to isolate or remove a particular fraction of the multimer of the homodimer in addition to polishing.

23. The method of claim 22, wherein the final protein titer of the composition is >4 g/L.

24. The method of claim 22, wherein the composition comprises >70% multimers.

25. The method of claim 1, further comprising shifting the pH from 7.1 to 7.0 at day 5.

26. The method of claim 7, further comprising shifting the pH from 7.1 to 7.0 at day 5.

27. The method of claim 7, wherein the final protein titer is at least 7.2 g/L.

28. A method for producing a composition comprising a homodimer and/or a multimer of the homodimer, wherein the homodimer comprises two monomers each comprising amino acids 21-264 of SEQ ID NO:4, the method comprising:

- (a) culturing Chinese Hamster Ovary (CHO) cells, that have been stably transfected with an expression vector comprising a nucleic acid sequence encoding SEQ ID NO: 4, at a growth temperature of $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. until the CHO cells reach a cell density of about 5 to about 30 million cells/mL;
- (b) shifting the growth temperature from $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. to $32.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$.; and
- (c) harvesting the homodimer and/or the multimer of the homodimer from the culture media.