

We Claim

1. A method of manufacturing pharmaceutical antigen binding protein with high yield and minimum aggregation, wherein the antigen binding protein is an anti-Dengue monoclonal antibody according to SEQ ID 1 and SEQ ID 2 or wherein the antigen binding protein is an anti-Rabies monoclonal antibody according to SEQ ID 3 and SEQ ID 4, the method comprising the steps of:
 - a. culturing in large scale mammalian cells that express antigen binding protein in a chemically defined, non-animal origin cell culture production medium, wherein the culturing step effectively maintains the cell count in the range of $10 \times 10^6 - 20 \times 10^6$ cells/ml and results in a yield of at least 2 g/L, wherein the cell line of mammalian cells is CHO-K1 SV GS-KO when the antigen binding protein is an anti-Dengue monoclonal antibody and the cell line of mammalian cells is GS-CHO when the antigen binding protein is an anti-Rabies monoclonal antibody; wherein the culturing step includes use of basal medium, use of concentrated basal medium as feed solution, use of feed solution along with a definite feeding strategy, resulting in enhanced cell growth, maintaining lower concentrations of lactate and ammonia, and effectively maintaining the cell count thereby increasing cell longevity and high yield;
 - b. purifying antigen binding protein from harvested supernatant obtained in step (a), wherein the purification results in recovery of at least 80%, purity of at least 99% and wherein the purification comprises affinity chromatography, Low pH viral inactivation, cation exchange chromatography, anion exchange chromatography, nanofiltration, Tangential flow filtration/Ultrafiltration; in a sequential manner; wherein the affinity chromatography matrix is Protein A; and wherein the salt concentration of the buffers used in purification is in the range of 30 mM – 500 mM;
 - c. preparing a stable formulation comprising the antigen binding protein, wherein osmolality of the formulation is in range of 300 - 400 mOsm/Kg and viscosity of the formulation is less than 2.5 mPa-S, wherein the formulation comprises at least one antigen binding protein, at least one stabilizer, at least one buffering

agent, at least one tonicity agent, and at least one surfactant, wherein the formulation comprises:

- i. 1-100 mg/ml of the antigen binding protein;
- ii. 20-40 mM of histidine;
- iii. 50-100 mM of arginine;
- iv. 0.002-0.02 % polysorbate 80 (w/v);
- v. 50-150 mM NaCl; and
- vi. 0.1-2.5% sucrose (w/v);

wherein the resulting formulation is stable at 2-8 °C for at least 9 months, at 25 °C for at least 1 month; at 40 °C for at least 40 days and at 50 °C for at least 2 days.

2. The method of claim 1, wherein the cell culture production medium is supplemented with one or more other nutrients, at least once during the cell culturing step.
3. The method of claim 1, wherein the cell culture production medium is supplemented on a schedule comprising supplementation that is continuous, daily, every other day, every two days and a combination thereof.
4. The method of claim 1, wherein the cell culture medium has an osmolality in range of 250 - 500mOsm/Kg; pH in range of 6.5 - 7.5; dissolved oxygen is maintained in range of 10 - 60%; Cell culture temperature is in the range of 30°C to 38°C; first temperature preferably 36 - 37 °C and optionally second temperature preferably 30 - 35 °C; glucose concentration is maintained below 7%; preferably between 4% and 5%; harvesting the cell culture when viability is decreased to 80 %; wherein the cell culture conditions are maintained in a manner that secondary metabolites including lactate concentration is not more than 5g/L; and ammonia concentration is not more than 5 mMol/L.
5. The method of claim 4, wherein the osmolality of the cell culture production medium is 400 – 500 mOsm/kg.

6. The method of claim 1, wherein the dissolved oxygen of the cell culture production medium is maintained in the range of 20 - 40%.
7. The method of claim 1, the cells are cultivated in a batch, fed batch, continuous mode, perfusion mode; more particularly in a fed batch mode.
8. The method of claim 1, wherein the salt concentration of the buffers used in purification is in the range of 50 mM - 300 mM.
9. The method of claim 1, further comprising an additional chromatography step selected from the group comprising one or more of Hydrophobic interaction chromatography, Hydrophobic charge induction chromatography, Ceramic hydroxyapatite chromatography, Multimodal chromatography, and Membrane chromatography.
10. The method of claim 1, wherein the protein A chromatography comprises:
 - a. Equilibration buffer: 20 mM Phosphate Buffer; 100 - 150 mM NaCl; 0.05% Polysorbate 80; pH 7.0 ± 0.2 ;
 - b. Loading : Clarified Harvest;
 - c. Wash I Buffer: 20 mM Phosphate buffer; 100 - 150 mM NaCl, more particularly 150mM; 0.05% Polysorbate 80; pH 7.0 ± 0.2 ;
 - d. Wash II Buffer: 20 mM Phosphate buffer; 250 mM - 1 M NaCl, more particularly 1M; 0.05% Polysorbate 80; pH 7.0 ± 0.2 ;
 - e. Wash III Buffer: 10 mM Phosphate buffer; 100 - 150 mM NaCl, more particularly 125mM; 0.05% Polysorbate 80; pH 7.0 ± 0.2 ;
 - f. Elution Buffer: 20mM Citrate buffer; pH 3.0 ± 0.2 ; and optionally 0.025 % (w/v) Polysorbate 80;
 - g. CIP Buffer: 0.1 M NaOH;
 - h. Residence time: 4.00 - 8.00 minutes;
 - i. Column used: XK26; and
 - j. Linear flow rate is 10 - 500 cm/hr, more particularly 100-150 cm/hr.

11. The method of claim 1, wherein viral inactivation of the eluate from protein A chromatography is accomplished by holding the eluate at pH 3.3 - 3.5 for a period of 50 - 100 minutes.
12. The method of claim 1, wherein the cation exchange chromatography comprises of resin selected from the group comprising one or more of sulfonate based group; a sulfoethyl based group; a sulphopropyl based group; a sulfoisobutyl based group; a sulfoxy ethyl based group, a carboxymethyl based group; sulfonic and carboxylic acid based groups; a carboxylic acid based group; a sulfonic acid based group; and an orthophosphate based group.
13. The method of claim 1, wherein the cation exchange chromatography comprises:
 - a. Pre-equilibration buffer: 200 mM Citrate buffer; pH 6.0 ± 0.2 ;
 - b. Equilibration buffer: 10 mM Citrate buffer; 0.025 % (w/v) Polysorbate 80; pH 6.0 ± 0.2 ;
 - c. Wash Buffer A: 10 mM Citrate buffer; pH 6.0 ± 0.2 ;
 - d. Wash buffer B: 20 mM Citrate buffer; 300 - 500 mM NaCl; pH 6.0 ± 0.2 ;
 - e. CIP buffer: 0.5M NaOH;
 - f. Residence time: 4.00 - 7.00 minutes; and
 - g. Column used: XK26.
14. The method of claim 1, wherein the anion exchange chromatography comprises of
 - a. Cleaning buffer: 0.5M NaOH;
 - b. Pre-equilibration buffer: 200 mM Citrate buffer; pH 6.0 ± 0.2 ;
 - c. Equilibration buffer: 20 mM Citrate buffer; pH 6.0 ± 0.2 ; and optionally 0.025% Polysorbate 80;
 - d. Storage buffer: 0.1 M NaOH;
 - e. Linear Flow rate is 10 - 500 cm/hr, more particularly 100-150 cm/hr; and
 - f. Column used: XK26.

15. The method of claim 1, wherein the anion exchange chromatography is "flow through and wash mode" or "bind and elute mode".
16. The method of claim 1, wherein the removal of viral particles is accomplished by nanofiltration using virus retentive filter.
17. The method of claim 1, wherein the antigen binding protein is concentrated using Tangential Flow Filtration (TFF).
18. The method of claim 17, wherein the TFF is carried out using 30 kDa membrane.
19. The method of claim 24, wherein the Tangential Flow Filtration process comprises:
 - a. Diafiltration using diafiltration buffer: 25 mM Histidine buffer; 75 mM Arginine buffer; 50 - 150 mM NaCl; pH 6.50 ± 0.5 ;
 - b. Cleaning buffer: 0.5M NaOH;
 - c. Storage buffer: 0.1 M NaOH;
 - d. Equilibration using 5 - 10 X membrane volume;
 - e. Concentration and Diafiltration using 10 - 20 diafiltration volume;
 - f. WFI wash using 3 - 5 membrane volume;
 - g. cleaning using 0.5 - 1.0 M NaOH; and
 - h. Storage 0.1 M NaOH.
20. The method of claim 1, wherein the purified therapeutic protein preparation contains no greater than 2% aggregates, preferably less than 1% aggregates.
21. The method of claim 1, wherein the stable antigen binding protein formulation comprises of 1 mg/ml to 100 mg/ml of antigen binding protein.
22. The method according to any one of the preceding claims, wherein antigen binding protein formulation comprises not more than 3% aggregation, minimum amount of sub-visible particles and improved potency.

23. The method of claim 1, wherein the concentration of the antigen binding protein monomer in the formulation is greater than 99%; residual CHO DNA is not more than 2 pg/mg of antigen binding protein, more particularly not more than 0.1 pg/mg of antigen binding protein; residual CHO protein is not more than 100 ng/mg of antigen binding protein, more particularly not more than 10 ng/mg of antigen binding protein; residual Protein-A is not more than 10 ng/mg of antigen binding protein, more particularly not more than 1.5 ng/mg of antigen binding protein; and Endotoxin is not more than 0.1 EU/mg of antigen binding protein.

24. The method of claim 1, wherein the formulation comprises:

- a. <1% (w/v), preferably 0.5% (w/v), sucrose;
- b. 25 mM, histidine;
- c. 75 mM, arginine;
- d. 100 – 145 mM, sodium chloride;
- e. 0.02%(w/v), Polysorbate 80; and
- f. 1 mg/ml to 50 mg/ml, antigen binding protein.

25. A pharmaceutical formulation comprising:

- a. 1 - 100 mg/ml of at least one antigen binding protein, wherein the antigen binding protein is an anti-Dengue monoclonal antibody according to SEQ ID 1 and SEQ ID 2 or wherein the antigen binding protein is an anti-Rabies monoclonal antibody according to SEQ ID 3 and SEQ ID 4;
- b. 20 - 40 mM of Histidine;
- c. 50 - 100 mM of Arginine;
- d. 0.002 - 0.02% Polysorbate 80 (w/v);
- e. 50 - 150 mM NaCl; and
- f. 1-2.5% sucrose w/v; wherein pH of the formulation is 6.5 ± 0.5 . wherein the osmolality of the formulation is 300 - 450 mOsmol/kg and viscosity less than 2.5 mPa-S and said formulation is stable at 2-8 °C for at least 9 months,

at 25 °C for at least 1 month, at 40 °C for at least 40 days, at 50 °C for at least 2 days.

26. The pharmaceutical formulation according to claim 25 comprising 2 - 80 mg/ml of at least one antigen binding protein; 25 mM of histidine; 75 mM of arginine; 101 mM NaCl; 0.02% polysorbate 80 (w/v); and 0.5% sucrose w/v; wherein pH of the formulation is 6.5 ± 0.5 .
27. The pharmaceutical formulation according to any one of claims 25 to 26, wherein the osmolality of the formulation is 380 mOsm/kg.
28. The pharmaceutical formulation according to any one of claims 25 to 27 comprising 2 - 80 mg/ml of antigen binding protein; 25 mM of Histidine; 75 mM of Arginine; 101 mM NaCl; 0.02% Polysorbate 80 (w/v); and 0.5% Sucrose w/v; wherein pH of the formulation is 6.5 ± 0.5 Osmolality 380 mOsm/Kg, viscosity less than 2.5 mPa-S.
29. The pharmaceutical formulation according to any one of claims 25 to 28 comprising 25 mg/ml of antigen binding protein antibody; 25 mM of Histidine; 75 mM of Arginine; 101 mM NaCl; 0.02% Polysorbate 80 (w/v); and 0.5% Sucrose w/v; wherein pH of the formulation is 6.5 ± 0.5 ; Osmolality is 380 mOsm/Kg, and viscosity is less than 2.5 mPa-S.
30. The pharmaceutical formulation according to any one of claims 25 to 28 comprising 50 mg/ml of antigen binding protein; 25 mM of Histidine; 75 mM of Arginine; 101 mM NaCl; 0.02% Polysorbate 80 (w/v); and 0.5% Sucrose w/v; wherein pH of the formulation is 6.5 ± 0.5 ; Osmolality is 380 mOsm/Kg, and viscosity is less than 2.5 mPa-S.
31. The pharmaceutical formulation according to any one of the preceding claims 25-30 wherein the formulation is a liquid formulation.

32. A pharmaceutical composition according to any one of claims 25 to 31 for use in the treatment, prevention or diagnosis of dengue or rabies virus.
33. The pharmaceutical formulation according to any one of the claims 25-32, wherein the formulation is comprised in a container selected from a bottle, a vial, an ampule, an IV bag, a wearable injector, a bolus injector, a syringe, a pen, a pump, a multidose needle syringe, a multidose pen, an injector, a syrette, an autoinjector, a pre-filled syringe, or a combination thereof.
34. The pharmaceutical formulation of claim 33, wherein at least one primary packaging component comprises a container closure selected from polypropylene (PP), polyethylene terephthalate (PETG), high-density polyethylene (HDPE), polyethylene terephthalate (PET), polypentafluorostyrene (PFS), polycarbonate, polyvinyl chloride (PVC), polycyclopentane (CZ.RTM.), cyclic olefin copolymer (COC), polyolefin, and combinations or copolymers thereof.