

Claims

- 1) A method of producing a heterodimeric antibody, comprising
 - a) providing a first homodimeric antibody comprising a first Fc region of an immunoglobulin comprising a first CH3 region and a second homodimeric antibody comprising a second Fc region of an immunoglobulin comprising a second CH3 region, wherein the amino acid sequences of the first CH3 region and the second CH3 regions are different and are such that the heterodimeric interaction between the first CH3 region and the second CH3 region is stronger than a homodimeric interaction between the first CH3 region or a homodimeric interaction between the second CH3 region;
 - b) combining the first homodimeric antibody and the second homodimeric antibody into a mixture;
 - c) incubating the mixture in the presence of a reducing agent; and
 - d) removing the reducing agent thereby producing the heterodimeric antibody, wherein the percentage (%) of dissolved oxygen (DO_2) is controlled to be 15% or lower in step d) or both in step c) and step d).
- 2) The method of claim 1, wherein the % DO_2 the mixture is 10% or less, 9% or less, 8% or less, 7% or less, 6% or less, 5% or less, 4% or less, 3% or less, 2% or less or 1% or less.
- 3) The method of claim 2, wherein % DO_2 is controlled by an overlay of nitrogen.
- 4) The method of claim 2 or 3, wherein the first homodimeric antibody and the second homodimeric antibody are combined into the mixture in step b) at a molar ratio of between 1:1 to 1:2.
- 5) The method of claim 2 or 3, wherein the first homodimeric antibody and the second homodimeric antibody are combined into the mixture in step b) at the molar ratio of 1.05:1.
- 6) The method of any one of claims 1-5, wherein total concentration of immunoglobulin in the mixture is 10.5 g/L.
- 7) The method of any one of claims 1-6, wherein the mixture is incubated in the presence of the reducing agent for 10 minutes or longer.
- 8) The method of any one of claims 1-7, wherein the mixture is incubated in the presence of the reducing agent from 10 minutes to 24 hours.
- 9) The method of any one of claims 1-8, wherein the reducing agent is 2-mercaptoethylamine (2-MEA), a chemical derivative of 2-MEA, L-cysteine or D-cysteine.
- 10) The method of claim 9, wherein concentration of 2-MEA in the mixture is 35 mM.
- 11) The method of any one of claims 1-10, wherein the mixture comprises a buffer.
- 12) The method of claim 11, wherein the buffer comprises a sodium acetate buffer.
- 13) The method of claim 12, wherein the buffer further comprises NaCl.

- 14) The method of claim 13, wherein the buffer comprises 100 mM sodium acetate and 30 mM NaCl.
- 15) The method of claim 14, wherein the pH of the buffer is 7.3.
- 16) The method of any one of claims 1-15, comprising a step of removing the reducing agent from the mixture.
- 17) The method of claim 16, wherein the reducing agent is removed by filtration.
- 18) The method of claim 17, wherein filtration is diafiltration.
- 19) The method of any one of claims 1-18, wherein the first homodimeric antibody and the second homodimeric antibody are an IgG1, IgG2 or IgG4 isotype.
- 20) The method of claim 19, wherein the first CH3 domain and the second CH3 domain comprise following mutations when compared to the wild-type IgG1 of SEQ ID NO: 1, wild-type IgG2 of SEQ ID NO: 2 or wild-type IgG4 of SEQ ID NO: 3: F405L/K409R, wild-type/F405L_R409K, T350I_K370T_F405L/K409R, K370W/K409R, D399AFGHILMNRSTVWY/K409R, T366ADEFHILMQVY/K409R, L368ADEGHNRSTVQ/K409AGRH, D399FHKRQ/K409AGRH, F405IKLSTVW/K409AGRH, Y407LWQ/K409AGRH, T366Y/F405A, T366W/F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S, T366W/T366S_L368A_Y407V, L351Y_F405A_Y407V/T394W, T366I_K392M_T394W/F405A_Y407V, T366L_K392M_T394W/F405A_Y407V, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, Y407A/T366A_K409F, T350V_L351Y_F405A_Y407V/T350V_T366L_K392L_T394W, K409D/D399K, K409E/D399R, K409D_K360D/D399K_E356K, K409D_K360D/D399E_E356K, K409D_K370D/D399K_E357K, K409D_K370D/D399E_E357K, K409D_K392D/D399K_E356K_E357K or K409D_K392D/D399E_E356K_E357K,
- 21) The method of claim 20, wherein the first Fc region and/or the second Fc region comprise one or more mutations when compared to the wild-type IgG1 of SEQ ID NO: 1, wild-type IgG2 of SEQ ID NO: 2 or wild-type IgG4 of SEQ ID NO: 3 that modulate binding of the first Fc region and/or the second Fc region to an Fc γ receptor (Fc γ R), an FcRn, or to protein A.
- 22) The method of claim 21, wherein the Fc γ R is Fc γ RI, Fc γ RIIa, Fc γ RIIb or Fc γ RIII.
- 23) The method of claim 21, wherein the one or more substitutions that modulate binding of the first Fc region and/or the second Fc region to the Fc γ are L234A_L235A, F234A_L235A, S228P_F234A_L235A, S228P_L234A_L235A, V234A_G237A_P238S_H268A_V309L_A330S_P331S, V234A_G237A, H268Q_V309L_A330S_P331S, S267E_L328F, L234F_L235E_D265A, L234A_L235A_G237A_P238S_H268A_A330S_P331S or S228P_F234A_L235A_G237A_P238S.

- 24) The method of claim 21, wherein the one or more substitutions that modulate binding of the first Fc region and/or the second Fc region to the FcRn are M428L_N434S, M252Y_S254T_T256E, T250Q_M428L, N434A and T307A_E380A_N434A, H435A, P257I_N434H, D376V_N434H, M252Y_S254T_T256E_H433K_N434F, T308P_N434A or H435R.
- 25) The method of claim 21, wherein the one or more substitutions that modulate binding of the first Fc region and/or the second Fc region to protein A are Q311R, Q311K, T307P_L309Q, T307P_V309Q, T307P_L309Q_Q311R, T307P_V309Q_Q311R, H435R or H435R_Y436F.
- 26) The method of any one of claims 1-25, wherein the steps of producing the heterodimeric antibody are conducted under GMP-compliant conditions.
- 27) The method of any one of claims 1-25, wherein the steps of producing the heterodimeric antibody are conducted during manufacture of a drug substance comprising the heterodimeric antibody.
- 28) The method of any one of claims 1-27, wherein the heterodimeric antibody is a bispecific antibody.
- 29) The method of claim 28, wherein the bispecific antibody binds CD3, CD123, BCMA, EGFR or c-Met, or any combination thereof.
- 30) The method of any one of claims 1-29, wherein a mass ratio of the total immunoglobulin to the reducing agent in the mixture is between 1.0 and 5.0.
- 31) The method of claim 30, wherein the mass ratio is between 1.4 and 3.8.
- 32) The method of claim 30, wherein the mass ratio is between 3.3 and 4.4.