Claims

1. A process for purifying a sample of h38C2 antibody or variant thereof, wherein both antigen binding sites are fully available for antigen binding in at least about 85% of the antibodies in the sample, comprising

   (i) Equilibrating a HIC column with a pre-loading equilibration wash comprising a base buffer that comprises between about 15mM and about 100mM sodium phosphate, potassium phosphate or ammonium phosphate HEPES, Tris and bis-Tris, at between about pH6.5 to about 7.5, and further comprises a salt selected from the group consisting of NaCl, KCl, and monosodium citrate, at a first concentration of between about 0.5M and 1.5M; wherein the HIC column comprises phenyl conjugated resin beads below about 50µm in diameter and comprising pores of at least about 500Å;

   (ii) Loading the column with a sample of h38C2 at between about 4 and about 80g/L in loading buffer comprising the base buffer and further comprising the salt at the first concentration;

   (iii) Washing the column with post-loading equilibration wash comprising the base buffer and the salt at the first concentration;

   (iv) Washing the column with a salt gradient, comprising the base buffer and further comprising a linear concentration gradient from about 1.5M to about 0.25M of the salt, characterised in that the salt concentration decreases by between about 90mM and 100mM per column volume (1CV);

   (v) Washing the column with a salt plateau wash, comprising between about 4CV and about 8CV of the salt at between about 0.25M and about 0.4M in the base buffer;

   (vi) Washing the column with a buffer wash comprising the base buffer;

   (vii) Eluting the h38C2 with an elution buffer, comprising the base buffer and a linear concentration gradient of 1,6 hexanediol beginning at a concentration of between about 0 to about 1% of 1,6 hexanediol and ending at an upper limit selected from the group consisting of about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, and 22% about 0% to about 22%

of 1,6 hexanediol for between about 0.5CV to about 3CV or until the elution pool is collected.

(viii) Optionally running a further elution step comprising the base buffer and 1,6 hexanediol at a concentration selected from the group consisting of about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, and 22% about 0% to about 22%, for up to 5CV or until the elution pool is collected;

wherein the h38C2 antibody or variant thereof comprises a light chain variable region (V_L) comprising a V_L CDR1, V_L CDR2, and V_L CDR3 of the V_L sequence shown in SEQ ID NO:27; and preferably as set forth in SEQ ID NO:27, and a heavy chain variable region (V_H) comprising a V_H CDR1, V_H CDR2, and V_H CDR3 of the V_H sequence shown in SEQ ID NO:28, and preferably as set forth in SEQ ID NO:28.

2. The process as claimed in claim 1, wherein the antibody further comprises a light chain constant region at least 95% identical to one or more of SEQ ID NOs:78, 79, 80 and 81, and a heavy chain constant region at least 95% identical to SEQ ID NO:82.
3. The process as claimed in any one of the preceding claims, wherein the antibody is an IgG1, and may comprise SEQ ID NO:25 and 26.
4. The process as claimed in any one of the preceding claims, wherein HIC column comprises phenyl conjugated resin beads of about 35µM comprising pores of about 750Å.
5. The process as claimed in any one of the preceding claims, wherein the pre-loading equilibration wash of step (i) comprises about 20mM sodium phosphate, about 1M NaCl, about pH7.
6. The process as claimed in any one of the preceding claims, wherein at step (ii), the column is loaded with a sample of h38C2 at between about 15 and about 18g/L in about 20mM sodium phosphate at about pH7.
7. The process as claimed in any one of the preceding claims, wherein at step (iii), the post-loading equilibration wash comprises about 1 CV of 1M NaCl in about 20mM sodium phosphate at about pH7.

8. The process as claimed in any one of the preceding claims, wherein at step (iv), the NaCl gradient comprises 20mM sodium phosphate pH7, and wherein and the linear concentration gradient is from about 1M to about 0.33M of NaCl, and the salt concentration decreases by between about 90mM and 100mM per 1CV.
9. The process as claimed in any one of the preceding claims, wherein at step (iv), the linear salt gradient comprises at least about 4 CV.
10. The process as claimed in any one of the preceding claims, wherein the salt plateau wash of step (v) comprises about 5 CV about 0.33M of NaCl in about 20mM sodium phosphate at about pH 7.
11. The process as claimed in any one of the preceding claims, wherein the base buffer at step (vi) comprises about 2CV of 20mM sodium phosphate at about pH7.
12. The process as claimed in any one of the preceding claims, wherein the elution buffer of step (vii) comprises 20mM sodium phosphate pH7.
13. The process as claimed in any one of the preceding claims, wherein the linear concentration gradient of step (vii) comprises a linear concentration gradient of 1,6 hexanediol beginning at a concentration of between about 0 to about 1% of 1,6 hexanediol and ending at an upper limit selected from the group consisting of about 14%, about 15%, or about 16%, of 1,6 hexanediol for about 1CV.
14. The process as claimed in any one of the preceding claims, wherein the further elution step comprises 20mM sodium phosphate pH7 and 1,6 hexanediol at a concentration selected from the group consisting of about 14%, about 15%, and about 16%, for about 2 to about 5CV or until the elution pool is collected.
15. A composition comprising h38C2 prepared according to any one of the preceding claims.

Claims

1. A process for purifying a sample of h38C2 antibody or variant thereof, wherein both antigen binding sites are fully available for antigen binding in at least about 85% of the antibodies in the sample, comprising

   (a) Equilibrating a hydrophobic interaction chromatography (HIC) column with a pre-loading equilibration wash comprising a base buffer that comprises between about 15mM and about 100mM sodium phosphate, potassium phosphate or ammonium phosphate HEPES, Tris and bis-Tris, at between about pH6.5 to about 7.5, and further comprises a salt selected from the group consisting of NaCl, KCl, and monosodium citrate, at a first concentration of between about 0.5M and 1.5M; wherein the HIC column comprises phenyl conjugated resin beads below about 50µm in diameter and comprising pores of at least about 500Å;

   (b) Loading the column with a sample of h38C2 at between about 4 and about 80g/L in loading buffer comprising the base buffer and further comprising the salt at the first concentration;

   (c) Washing the column with post-loading equilibration wash comprising the base buffer and the salt at the first concentration;

   (d) Washing the column with a salt gradient, comprising the base buffer and further comprising a linear concentration gradient from about 1.5M to about 0.25M of the salt, characterised in that the salt concentration decreases by between about-90mM and 100mM per 1 column volume (CV);

   (e) Washing the column with a salt plateau wash, comprising between about 4CV and about-8CV of the salt at between about 0.25M and about 0.4M in the base buffer;

   (f) Washing the column with a buffer wash comprising the base buffer;

   (g) Eluting the h38C2 with an elution buffer, comprising the base buffer and a linear concentration gradient of 1,6 hexanediol beginning at a concentration of between about 0 to about 1% of 1,6 hexanediol and ending at an upper limit selected from the group consisting of about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, and 22% about 0% to about 22% of 1,6 hexanediol for between about 0.5CV to about 3CV or until the elution pool is collected,

(h) Optionally running a further elution step comprising the base buffer and 1,6 hexanediol at a concentration selected from the group consisting of about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, about 21%, and 22% about 0% to about 22%, for up to 5CV or until the elution pool is collected;

wherein the h38C2 antibody or variant thereof is an IgG1 antibody comprising a light chain variable region (V_L) as set forth in SEQ ID NO:27; and a heavy chain variable region (V_H) as set forth in SEQ ID NO:28.

2. The process as claimed in claim 1, wherein the antibody further comprises a light chain constant region at least 95 percent identical to one or more of SEQ ID NOs:78, 79, 80 and 81, and a heavy chain constant region at least 95 percent identical to SEQ ID NO:82.

3. The process as claimed in claim 1, wherein the antibody is an IgG1, and may comprise SEQ ID NO:25 and 26.

4. The process as claimed in claim 1, wherein HIC column comprises phenyl conjugated resin beads of about 35 micro M comprising pores of about 750 Å.

5. The process as claimed in claim 1, wherein the pre-loading equilibration wash of step (a) comprises about 20 mM sodium phosphate, about 1M NaCl, about pH7.

6. The process as claimed in claim 1, wherein at step (b), the column is loaded with a sample of h38C2 at between about 15 and about 18 g/L in about 20 mM sodium phosphate at about pH7.

7. The process as claimed in claim 1, step (c), the post-loading equilibration wash comprises about 1 CV of 1M NaCl in about 20 mM sodium phosphate at about pH7.

8. The process as claimed in claim 1, wherein at step (d), the salt gradient comprises 20 mM sodium phosphate pH7, and wherein and the linear concentration gradient is from about 1M to about 0.33M of NaCl, and the salt concentration decreases by between about 90 mM and 100 mM per 1 CV.

9. The process as claimed in claim 1, wherein at step (d), the linear salt gradient comprises at least about 4 CV.

10. The process as claimed in claim 1, wherein the salt plateau wash of step (e) comprises about 5 CV and about 0.33M of NaCl in about 20 mM sodium phosphate at about pH 7.

11. The process as claimed in claim 1, wherein the base buffer at step (f) comprises about 2CV of 20 mM sodium phosphate at about pH7.

12. The process as claimed in claim 1, wherein the elution buffer of step (g) comprises 20 mM sodium phosphate pH7.

13. The process as claimed in claim 1, wherein the linear concentration gradient of step (g) comprises a linear concentration gradient of 1,6 hexanediol beginning at a concentration of between about 0 to about 1 percent of 1,6 hexanediol and ending at an upper limit selected from the group consisting of about 14 percent, about 15 percent, or about 16 percent, of 1,6 hexanediol for about 1 CV.

14. The process as claimed in claim 1, wherein the further elution step of step (h) comprises 20 mM sodium phosphate pH7 and 1,6 hexanediol at a concentration selected from the group consisting of about 14 percent, about 15 percent, and about 16 percent, for about 2 to about 5CV or until the elution pool is collected.