

CLAIMS

1. A variant IgG4 antibody which has been modified in the heavy chain constant region, wherein the variant IgG4 antibody has a reduced level of binding to host cell protein (HCP), compared to an unmodified IgG4 antibody, wherein the modification comprises:
 - (a) modification of Kabat residue 203; and/or
 - (b) modification of Kabat residue 222; and/or
 - (c) substitution of one or more amino acids comprising K203 to R, E, or Q; R222 to T, K, or Q; E226 to L or I; S227 to R, A, N, or T; Y229 to S, F, W, or H; G230 to A, N, or S; P237 to H, E, D, or V; and/or P238 to T, K, or E; and/or
 - (d) replacement of ESKYGPP (SEQ ID NO: 26) (Kabat residues 226 to 238) with EPKSCDKTHT (SEQ ID NO: 27), or ERKYGPP (SEQ ID NO: 28), or ERKCCVE (SEQ ID NO: 29), or ELKTPLGDTTHT (SEQ ID NO: 30); and/or
 - (e) replacement of YGPP (SEQ ID NO: 23) (Kabat residues 229 to 238) with SCDKTHT (SEQ ID NO: 24), or CCVE (SEQ ID NO: 25).
2. The variant IgG4 antibody according to claim 1, wherein the modification comprises substitution of R222 to K.
3. The variant IgG4 antibody according to claim 1 or claim 2, wherein the modification comprises replacement of YGPP (SEQ ID NO: 23) (Kabat residues 229 to 238) with CCVE (SEQ ID NO: 25).
4. The variant IgG4 antibody according to any one of claims 1 to 3, wherein the IgG4 antibody has reduced binding affinity and/or binding activity to host cell protein (HCP) in comparison to the unmodified IgG4 antibody.
5. The variant IgG4 antibody according to any one of claims 1 to 4, wherein the variant IgG4 antibody comprises a further substitution of S241 to P and/or L248 to E and/or replacement of EFLGGP (SEQ ID NO: 31) (Kabat residues 246 to 251) with PAAAP (SEQ ID NO: 33) or PAAAS (SEQ ID NO: 32).
6. The variant IgG4 antibody according to any one of claims 1 to 5, wherein the variant IgG4 antibody comprises the sequence CPPC (SEQ ID NO: 20) (Kabat residues 239 to 242).

7. The variant IgG4 antibody according to any one of claims 1 to 6, wherein no further modifications are made in the heavy chain constant region in comparison to the unmodified IgG4 antibody.
8. The variant IgG4 antibody according to any one of claims 1 to 7, wherein the host cell protein is putative phospholipase B-Like 2 (PLBL2).
9. A cell line encoding the variant IgG4 antibody of any one of claims 1 to 8.
10. A method of modifying an IgG4 antibody to reduce binding to a process impurity, wherein the process impurity is a host cell protein, comprising the steps of:
 - a) identifying at least one amino acid involved in the binding of the process impurity using a method for studying protein-protein interactions, wherein the method for studying protein-protein interactions is hydrogen deuterium exchange mass spectrometry (HDX-MS), crystallography, Yeast 2-Hybrid screening, in silica 3D structure modelling or any combination thereof; and
 - b) creating a variant of the IgG4 antibody by substituting the one or more amino acid identified as being involved in the binding with the process impurity with a different amino acid.
11. The method according to claim 10, wherein the method for studying protein-protein interactions is hydrogen deuterium exchange mass spectrometry (HDX-MS).
12. The method according to claim 10 or claim 11, wherein the amino acid identified as being involved in the binding with the process impurity is present in the heavy chain constant region.
13. The method according to any one of claims 10 to 12, wherein the one or more amino acid identified as being involved in the binding with the process impurity is substituted with an amino acid residue at the corresponding position from an IgG1, IgG2, and/or IgG3 antibody germline sequence.
14. The method according to any one of claims 10 to 13, wherein the one or more amino acid identified as being involved in the binding with the process impurity is one or more of the following amino acid residues: lysine at position 203 (K203), arginine at position 222 (R222), glutamate at position 226 (E226), serine at position 227 (S227), tyrosine at position 229 (Y229), glycine at position 230 (G230), proline at position (P237), proline at position 238

(P238), ESKYGPP (SEQ ID NO: 26) (Kabat residues 226 to 238) and YGPP (SEQ ID NO: 23) (Kabat residues 229 to 238).

15. A method of producing an IgG4 antibody with reduced binding to host cell protein (HCP) comprising modifying the antibody sequence in the heavy chain constant region, wherein the modification comprises:

- (a) modification of Kabat residue 203; and/or
- (b) modification of Kabat residue 222; and/or
- (c) substitution of one or more amino acids comprising K203 to R, E, or Q; R222 to T, K, or Q; E226 to L or I; S227 to R, A, N, or T; Y229 to S, F, W, or H; G230 to A, N, or S; P237 to H, E, D, or V; and/or P238 to T, K, or E; and/or
- (d) replacement of ESKYGPP (SEQ ID NO: 26) (Kabat residues 226 to 238) with EPKSCDKTHT (SEQ ID NO: 27), or ERKYGPP (SEQ ID NO: 28), or ERKCCVE (SEQ ID NO: 29), or ELKTPLGDTTHT (SEQ ID NO: 30); and/or
- (e) replacement of YGPP (SEQ ID NO: 23) (Kabat residues 229 to 238) with SCDKTHT (SEQ ID NO: 24), or CCVE (SEQ ID NO: 25).

16. The method according to any one of claims 10 to 15, wherein the host cell protein is putative phospholipase B-Like 2 (PLBL2).

17. A composition comprising the variant IgG4 antibody according to any one of claims 1 to 8.

18. The composition according to claim 17, wherein the variant IgG4 antibody is at a concentration of at least 10mg/mL, at least 20 mg/mL, at least 50mg/mL, at least 75mg/mL or at least 100mg/mL.

19. The composition according to claim 17 or claim 18, wherein the concentration of putative phospholipase B-Like 2 (PLBL2) in the composition is less than 500ppm, less than 100ppm or less than 10ppm.

20. The composition according to any one of claims 17 to 19, which additionally comprises a buffer and/or a fatty acid ester; optionally wherein the fatty acid ester is Polysorbate 20 or Polysorbate 80.