

Patent Claims

1. A method for producing an antibody of the human IgG1 subclass by cultivating a CHO cell that has been transfected with one or more expression cassettes comprising the nucleic acids encoding the heavy and the light chains of the antibody,
5 wherein the nucleic acids encoding the antibody heavy and light chains are codon usage optimized with the codon usage of human cells and/or the codon usage of CHO cells,
10 wherein in the part of the nucleic acid encoding the heavy chain variable domain at least one non-paired donor splice site is removed.
2. The method according to claim 1, wherein in the part of the nucleic acid encoding the heavy chain constant region non-paired donor splice sites are not removed.
3. The method according to any one of claims 1 to 2, wherein in the nucleic acid encoding the antibody light chain non-paired splice sites are removed.
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4. The method according to any one of claims 1 to 3, wherein the transfection is a transient transfection.
5. The method according to any one of claims 1 to 4, wherein the nucleic acids encoding the antibody are cDNA.
- 20 6. The method according to any one of claims 1 to 4, wherein the nucleic acids encoding the light or/and the heavy chain of the antibody are genomically organized DNA.
7. The method according to any one of claims 1 to 6, wherein the removal of non-paired splice sites is by removal of non-paired donor splice sites by introducing amino acid sequence silent nucleotide changes in the non-paired donor splice site nucleic acid sequence.
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8. The method according to any one of claims 1 to 7, wherein the removal of the non-paired splice sites is by introducing an amino acid silent mutation in the nucleotide sequence NGGTA(G)AG (SEQ ID NO: 01).

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9. The method according to any one of claims 1 to 8, wherein the removal of the non-paired splice sites is by introducing an amino acid silent mutation in the nucleotide sequence NGGTA(G)AG (SEQ ID NO: 01) in the codon NGG or the codon GGT or the codon GTA(G).
- 5 10. The method according to any one of claims 1 to 9, wherein the method comprises the following steps:
- a) cultivating the CHO cell, and
- b) recovering the antibody from the CHO cell or the cultivation medium.
- 10 11. A method for producing an antibody of the human IgG1 subclass by cultivating a CHO cell comprising one or more nucleic acids encoding the heavy and the light chains of the antibody,
- 15 wherein in the nucleic acid encoding the heavy chain variable domain non-paired donor splice sites are removed by introducing amino acid sequence silent nucleotide changes in the non-paired donor splice site consensus sequence NGGTA(G)AG (SEQ ID NO: 01) in the codon NGG or the codon GGT or the codon GTA(G),
- wherein non-paired donor splice sites according to the sequence of SEQ ID NO: 01 are not removed in the nucleic acid encoding the heavy chain constant region.
- 20 12. The method according to claim 11, wherein the one or more nucleic acids encoding the antibody heavy and light chains are genomically organized DNA.
13. The method according to any one of claims 11 to 12, wherein the one or more nucleic acids encoding the antibody heavy and light chains are codon usage optimized with the codon usage of human cells and/or the codon usage of CHO cells.
- 25 14. The method according to any one of claims 11 to 13, wherein in the nucleic acid encoding the antibody light chain non-paired splice sites are removed by introducing amino acid sequence silent nucleotide changes in the non-paired donor splice site consensus sequence NGGTA(G)AG (SEQ ID NO: 01) in the codon NGG or the codon GGT or the codon GTA(G).
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15. Use of the removal of non-paired donor splice sites in a part of a human or hamster codon-usage optimized nucleic acid sequence encoding an antibody, whereby the part is the part that encodes the heavy chain variable domain, for reducing mis-splicing or/and increasing antibody expression yield when said nucleic acid is used to produce the antibody in CHO cells.
16. The use according to claim 15, wherein non-paired splice sites are not removed in the part of the nucleic acid encoding the heavy chain constant region.
17. The use according to any one of claims 15 to 16, wherein in the part of the nucleic acid encoding the light chain non-paired splice sites are removed.
18. The use according to any one of claims 15 to 17, wherein the removal of the non-paired splice sites is by introducing an amino acid silent mutation in the nucleotide sequence NGGTA(G)AG (SEQ ID NO: 01).
19. The use according to any one of claims 15 to 18, wherein the removal of the non-paired splice sites is by introducing an amino acid silent mutation in the nucleotide sequence NGGTA(G)AG (SEQ ID NO: 01) in the codon NGG or the codon GGT or the codon GTA(G).
20. The method or the use according to any one of claims 1 to 19, wherein the non-paired splice site is an artificial non-paired splice site.
21. The method or the use according to any one of claims 1 to 19, wherein the non-paired donor splice site is an artificial donor splice site and has been generated during codon optimization.