Claims

1. A method of producing at least two antibodies comprising
   - providing cells with nucleic acid that encodes the antibodies;
   - culturing said cells;
   - collecting the antibodies from the culture; and
   - separating produced antibodies from half antibodies by ion exchange chromatography (IEX);

the method characterized in that the antibodies exhibit IEX retention times that that deviate by 10% or less from the average of the retention times of the individual antibodies under the IEX conditions used.

2. The method of claim 1, wherein the collection of the antibodies from the culture comprises purifying antibody from other proteins by antibody affinity purification, preferably by protein A extraction.

3. The method of claim 2, wherein further comprising subjecting affinity purified antibodies to size-exclusion chromatography (gel-filtration chromatography and/or anion-exchange chromatography.

4. The method of claims 1-3, wherein subsequent to the IEX the collected antibodies are quantitatively analyzed for relative expression levels by hydrophobic interaction chromatography (HIC).

5. The method of claim 4, wherein the specificity of the collected antibodies is verified by ELISA.

6. The method of claims 1-5, wherein the retention times of the respective half antibodies are outside the range spanned by the retention times of the antibodies.

7. The method of claims 6, wherein the cells produce 3 heavy chains.

8. The method of claim 7, wherein said heavy chains comprise domains for efficient heterodimerization of heavy chains.

9. The method of claim 1-8, wherein at least two of said antibodies are bispecific antibodies.

10. The method of claim 1-9, wherein at least two of said antibodies share an identical heavy chain.

11. The method of claims 1-10, wherein said antibodies have isoelectric points (pI) that differ by 0.4 units or less from the average pI of said at least two antibodies.

12. The method of claims 1-11, wherein the antibodies are selected for having heavy and light chain combinations that have retention times that are significantly different from the retention times of the full antibodies under the IEX conditions used.

13. The method of claim 12, wherein the pI of heavy and light chain combinations differ by more than 0.4 units from the average pI of said at least two antibodies.

14. The method of claims 1-13, wherein the heavy chains comprise a CH3 domain that favors heterodimerization of heavy chains.

15. The method of claim 1-14, wherein the heavy chains of said antibodies are IgG heavy chains.

16. The method of claims 7-15, wherein one heavy chain comprises the amino acid substitutions L351K and T366K (EU numbering) in the CH3 region and another heavy chain comprises the amino acid substitutions L351D and L368E in the CH3 region.

17. A method of producing at least two antibodies comprising
- providing cells with nucleic acid that encodes the antibodies;
- culturing said cells;
- collecting the antibodies from the culture; and
- separating produced antibodies from half antibodies by ion exchange chromatography (IEX);

the method characterized in that the antibodies exhibit IEX retention times that that deviate by 10% or less from the average of the retention times of the individual antibodies under the IEX conditions used and wherein subsequent to the IEX the collected antibodies are quantitatively analyzed for relative expression levels by hydrophobic interaction chromatography (HIC) and the specificity of the collected antibodies is verified by ELISA.

18. A composition comprising 2-10 recombinant antibodies obtainable by a method of claims 1-17.

19. A composition comprising 2-10 recombinant antibodies characterized in that the IEX retention times of at least two of said antibodies deviate by 10% or less from the average of the retention times of the individual antibodies under the IEX conditions.

20. A composition comprising 2-10 recombinant antibodies characterized in that the pI of at least two of said antibodies differ by 0.4 units or less from the average pI of said at least two antibodies.

21. The composition of claims 18-20, characterized in that the IEX retention times and/or the pI are essentially the same for all of the antibodies.

22. The composition of claims 18-21, wherein at least two of the antibodies are bispecific antibodies.

23. The composition of claim 22, wherein at least two of said antibodies share an identical heavy chain.

Claims

1. A method of producing at least two multispecific antibodies comprising

- providing cells with nucleic acid that encodes the multispecific antibodies;
- culturing said cells;
- collecting the multispecific antibodies from the culture; and
- separating the produced multispecific antibodies from half antibodies by ion exchange chromatography (IEX);

the method characterized in that the antibodies exhibit IEX retention times that deviate by 10% or less from the average of the retention times of the individual antibodies under the IEX conditions used.

2. The method of claim 1, further comprising selecting at least two bispecific antibodies that have essentially the same IEX retention times under the conditions used for the IEX.

3. The method of claim 1 or 2, wherein the collection of the antibodies from the culture comprises purifying antibody from other proteins by antibody affinity purification, preferably by protein A extraction.

4. The method of claim 3, further comprising subjecting affinity purified antibodies to size-exclusion chromatography, gel-filtration chromatography and/or anion-exchange chromatography.

5. The method of any one of claims 1-4, wherein subsequent to the IEX the collected antibodies are quantitatively analyzed for relative expression levels by hydrophobic interaction chromatography (HIC).

6. The method of claim 5, wherein the specificity of the collected antibodies is verified by ELISA.

7. The method of any one of claims 1-6, wherein the retention times of the respective half antibodies are outside the range spanned by the retention times of the antibodies.

8. The method of claims 7, wherein the cells produce 3 heavy chains.

9. The method of claim 8, wherein said heavy chains comprise domains for efficient heterodimerization of heavy chains.

10. The method of any one of claim 1-9, wherein at least two of said antibodies are bispecific antibodies.

11. The method of any one of claim 1-10, wherein at least two of said antibodies share an identical heavy chain.

12. The method of any one of claims 1-11, wherein said antibodies have isoelectric points (pI) that differ by 0.4 units or less from the average pI of said at least two antibodies.

13. The method of any one of claims 1-12, wherein the antibodies are selected for having heavy and light chain combinations of the respective half antibodies that have retention times that are significantly different from the retention times of the full antibodies under the IEX conditions used.

14. The method of claim 13, wherein the pI of heavy and light chain combinations of the respective half antibodies differ by more than 0.4 units from the average pI of said at least two antibodies.

15. The method of any one of claims 1-14, wherein the antibodies have heavy chains comprising a CH3 domain that favors heterodimerization of heavy chains.

16. The method of any one of claim 1-15, wherein said antibodies have IgG heavy chains.

17. The method of any one of claims 8-16, wherein said cells produce two or more heavy chains wherein one heavy chain comprises the amino acid substitutions L351K and T366K (EU numbering) in the CH3 region and another heavy chain comprises the amino acid substitutions L351D and L368E in the CH3 region.

18. A method of producing at least two multispecific antibodies comprising

- providing cells with nucleic acid that encodes the multispecific antibodies;
- culturing said cells;
- collecting the multispecific antibodies from the culture; and
- separating the produced multispecific antibodies from half antibodies by ion exchange chromatography (IEX);

the method characterized in that the multispecific antibodies exhibit IEX retention times that deviate by 10% or less from the average of the retention times of the individual antibodies under the IEX conditions used and wherein subsequent to the IEX the collected multispecific antibodies are quantitatively analyzed for relative expression levels by hydrophobic interaction chromatography (HIC) and the specificity of the collected multispecific antibodies is verified by ELISA.

19. A composition comprising 2-10 recombinant multispecific antibodies wherein at least two of the antibodies are characterized in that the IEX retention times of at least two of said antibodies deviate by 10% or less from the average of the retention times of the individual antibodies under the IEX conditions.

20. A composition comprising 2-10 recombinant multispecific antibodies wherein at least two of the antibodies are characterized in that the pI of at least two of said antibodies differ by 0.4 units or less from the average pI of said at least two antibodies.

21. The composition of claim 19 or 20, characterized in that the IEX retention times and/or the pI are essentially the same for all of the antibodies.

22. The composition of any one of claims 19-21, wherein at least two of the antibodies are bispecific antibodies.

23. The composition of claim 22, wherein at least two of said antibodies share an identical heavy chain.