

wherein the first expression vector comprises exactly one nucleic acid sequence encoding a polypeptide of the multispecific antibody, and the one, two or three further expression vectors each comprise at least two nucleic acid sequences each encoding different polypeptide chains of the multispecific antibody,

wherein the exactly one nucleic acid sequence of the first expression vector is a nucleic acid sequence encoding a light chain polypeptide of the multispecific antibody,

- recovering the multispecific antibody from the cell or the cultivation medium,

and thereby producing the multispecific antibody; wherein the multispecific antibody comprises:

- a) a first light chain,
- b) a first heavy chain comprising a first heteromomeric Fc-region polypeptide,
- c) a second light chain, and
- d) a second heavy chain comprising a second heteromomeric Fc-region polypeptide.

As disclosed herein is a method for generating/producing/obtaining a mammalian cell (capable of (stably)) expressing a multispecific antibody, which comprises/is composed of at least three different polypeptides, comprising the following step:

- a) transfecting a mammalian cell (not expressing an antibody) with a first expression vector and one, two or three further expression vectors,

wherein the first expression vector comprises exactly one nucleic acid sequence encoding a polypeptide of the multispecific antibody,

Patent Claims

1. A method for producing a multispecific antibody, which comprises at least three different polypeptides, comprising the following steps:
 - cultivating a mammalian cell in a cultivation medium, whereby the mammalian cell has been generated by
 - a) transfecting a mammalian cell with a first expression vector and one, two or three further expression vectors,
wherein the first expression vector comprises exactly one nucleic acid sequence encoding a polypeptide of the multispecific antibody, and the one, two or three further expression vectors each comprise at least two nucleic acid sequences each encoding different polypeptide chains of the multispecific antibody,
wherein the exactly one nucleic acid sequence of the first expression vector is a nucleic acid sequence encoding a light chain polypeptide of the multispecific antibody,
 - recovering the multispecific antibody from the cell or the cultivation medium,
and thereby producing the multispecific antibody; wherein the multispecific antibody comprises:
 - a) a first light chain,
 - b) a first heavy chain comprising a first heteromomeric Fc-region polypeptide,
 - c) a second light chain, and
 - d) a second heavy chain comprising a second heteromomeric Fc-region polypeptide.
2. The method according to claim 1, wherein two of the polypeptide chains of the multispecific antibody comprise a domain exchange.

3. The method according to claim 2, wherein the exactly one nucleic acid of the first expression vector encodes a light chain polypeptide with domain exchange of the multispecific antibody.
- 5 4. The method according to any one of claims 1 to 3, wherein step a) is co-transfecting the first expression vector and the one, two or three further expression vectors.
5. The method according to any one of claims 1 to 3, wherein the mammalian cell is transfected first with the one, two or three further expression vectors and transfected thereafter with the first expression vector.
- 10 6. The method according to any one of claims 1 to 5, wherein the mammalian cell stably expresses the multispecific antibody.
7. The method according to any one of claims 1 to 6, wherein the mammalian cell is a CHO cell.
- 15 8. The method according to any one of claims 2 to 7, wherein the domain exchange is a CH1-CL crossover or a VH-VL-crossover.
9. The method according to any one of claims 1 to 8, wherein the multispecific antibody is a bivalent, bispecific antibody comprising
 - a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and
 - 20 b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the variable domains VL and VH of the second light chain and the second heavy chain are replaced by each other.
- 25 10. The method according to any one of claims 1 to 8, wherein the multispecific antibody is a bivalent, bispecific antibody comprising
 - a) a first light chain and a first heavy chain of an antibody specifically binding to a first antigen, and

- b) a second light chain and a second heavy chain of an antibody specifically binding to a second antigen, wherein the constant domains CL and CH1 of the second light chain and the second heavy chain are replaced by each other.

5 11. The method according to any one of claims 1 to 8, wherein the multispecific antibody is a trivalent, bispecific antibody, comprising

- a) a first light chain and a first heavy chain of a full length antibody which specifically binds to a first antigen,

10 b) a second heavy chain of a full length antibody which when paired with the first light chain, specifically binds to the first antigen, and

- c) a Fab fragment, which specifically binds to a second antigen, fused via a peptidic linker to the C-terminus of one of the heavy chains of a) or b), wherein the constant domains CL and CH1 of the second light chain and the second heavy chain are replaced by each other.

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