Enclosure to letter of July 16, 2015
International Application No. PCT/EP2015/057164
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Applicant's Ref.: P32624-WO (ASK)

Amended Claims

1. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the analysis of the multispecific antibody's light chain pairing, wherein the proteolytic enzyme is Lys-C, and wherein the incubating is for 35 to 45 minutes.

2. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the determination of light chain mispairing in the multispecific antibody, wherein the proteolytic enzyme is Lys-C, and wherein the incubating is for 35 to 45 minutes.

3. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody produced by a recombinant mammalian cell for the selection of a multispecific antibody producing mammalian cell, wherein the proteolytic enzyme is Lys-C, and wherein the incubating is for 35 to 45 minutes.

4. A method for the determination of the light chain pairing in a multispecific antibody comprising the following steps:

   a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for a limited time,

   b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and

   c) determining from the results in step b) the light chain pairing of the multispecific antibody,

   wherein the proteolytic enzyme is Lys-C, and

   wherein the incubating is for 35 to 45 minutes.

5. A method for the determination of light chain mispairing of a multispecific antibody comprising the following steps:

a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for a limited time,

b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and

c) determining from the results in step b) the light chain pairing of the multispecific antibody and thereby determining light chain mispairing,

wherein the proteolytic enzyme is Lys-C, and

wherein the incubating is for 35 to 45 minutes.

6. A method for the selection of a recombinant mammalian cell producing a multispecific antibody comprising the following steps:

a) individually incubating a sample comprising a multispecific antibody produced by a clonal population of a recombinant mammalian cell of a multitude of recombinant mammalian cells all cells of the multitude producing the same multispecific antibody with a proteolytic enzyme for a limited time,

b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry,

c) determining from the results in step b) the presence of light chain mispairing of the multispecific antibody for each clonal cell population, and

d) selecting based on the results in step c) a recombinant cell producing a multispecific antibody,

wherein the proteolytic enzyme is Lys-C, and

wherein the incubating is for 35 to 45 minutes.

7. The use or the method according to any one of claims 1 to 6, wherein the incubating is for about 40 minutes.

8. The use or the method according to any one of claims 1 to 7, wherein the multispecific antibody that is incubated with the proteolytic enzyme is a deglycosylated multispecific antibody.

9. The use or the method according to any one of claims 1 to 8, wherein the weight ratio of antibody to enzyme is about 1:200.

10. The use or the method according to any one of claims 1 to 9, wherein the multispecific antibody that is incubated with the proteolytic enzyme has a concentration of from 200 to 600 µg/mL.

11. The method according to any one of claims 4 to 10, wherein step b) is

    b) desalting the incubation mixture of step a) and identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry.

12. The use or the method according to any one of claims 1 to 11, wherein the multispecific antibody is a monoclonal multispecific antibody.

13. The use or the method according to any one of claims 1 to 12, wherein the multispecific antibody comprises at least two non-peptidically bound light chains.

14. The use or the method according to any one of claims 1 to 12, wherein the multispecific antibody comprises at least three non-peptidically bound polypeptides.

Patent Claims

1. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the analysis of the multispecific antibody's light chain pairing.

2. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody for the determination of light chain mispairing in the multispecific antibody.

3. Use of a limited digestion with a proteolytic enzyme of a multispecific antibody produced by a recombinant mammalian cell for the selection of a multispecific antibody producing mammalian cell.

4. A method for the determination of the light chain pairing in a multispecific antibody comprising the following steps:

   a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for a limited time,

   b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and

   c) determining from the results in step b) the light chain pairing of the multispecific antibody.

5. A method for the determination of light chain mispairing of a multispecific antibody comprising the following steps:

   a) incubating a sample comprising the multispecific antibody with a proteolytic enzyme for a limited time,

   b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry, and

   c) determining from the results in step b) the light chain pairing of the multispecific antibody and thereby determining light chain mispairing.

6. A method for the selection of a recombinant mammalian cell producing a multispecific antibody comprising the following steps:

a) individually incubating a sample comprising a multispecific antibody produced by a clonal population of a recombinant mammalian cell of a multitude of recombinant mammalian cells all cells of the multitude producing the same multispecific antibody with a proteolytic enzyme for a limited time,

b) identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry,

c) determining from the results in step b) the presence of light chain mispairing of the multispecific antibody for each clonal cell population, and

d) selecting based on the results in step c) a recombinant cell producing a multispecific antibody.

7. The use or the method according to any one of claims 1 to 6, wherein the proteolytic enzyme is selected from the group consisting of Lys-C, Asp-N, Arg-C, Glu-C and chymotrypsin.

8. The use or the method according to claim 7, wherein the proteolytic enzyme is Lys-C.

9. The use or the method according to any one of claims 1 to 8, wherein the incubating is for 35 to 45 minutes.

10. The use or the method according to claim 9, wherein the incubating is for about 40 minutes.

11. The use or the method according to any one of claims 1 to 10, wherein the multispecific antibody that is incubated with the proteolytic enzyme is a deglycosylated multispecific antibody.

12. The use or the method according to any one of claims 1 to 11, wherein the weight ratio of antibody to enzyme is about 1:200.

13. The use or the method according to any one of claims 1 to 12, wherein the multispecific antibody that is incubated with the proteolytic enzyme has a concentration of from 200 to 600 µg/mL.

14. The method according to any one of claims 4 to 13, wherein step b) is

b) desalting the incubation mixture of step a) and identifying the mass of the fragments obtained by the limited proteolytic digestion in step a) by mass spectrometry.

15. The use or the method according to any one of claims 1 to 14, wherein the multispecific antibody is a monoclonal multispecific antibody.

16. The use or the method according to any one of claims 1 to 15, wherein the multispecific antibody comprises at least two non-peptidically bound light chains.

17. The use or the method according to any one of claims 1 to 15, wherein the multispecific antibody comprises at least three non-peptidically bound polypeptides.