CLAIMS

1. A method for the manufacture of recombinant antibody molecules comprising

   a) culturing a host cell sample of gram-negative bacteria transformed with an expression vector encoding a recombinant antibody molecule;

   b) adding an extraction buffer to the sample; and

   c) subjecting the sample to a heat treatment step within a range of 40°C to 70°C for 1 to 24 hours;

   characterized in that the pH of the sample is detected after addition of the extraction buffer to ensure that the pH of the sample is 7 to 9 prior to the heat treatment step.

2. The method according to claim 1, wherein the pH is detected before and during the heat up phase.

3. The method according to claim 2, wherein the pH is lower during than before the heat up phase.

4. The method according to claim 1, 2 or 3, wherein pH of the sample is monitored continuously from the point of adding the extraction buffer to the start of heat the treatment step.

5. The method according to claim 4, wherein pH of the sample is monitored continuously during the treatment step.

6. The method according to any one of claims 1 to 5, wherein heat treatment step is performed within the range of 40°C to 65°C.

7. The method according to any one of claims 1 to 6, wherein the extraction buffer is selected from the group comprising NaOH, NH_4OH, sulphuric acid, EDTA, Tris buffer and combinations thereof.

8. The method according to any one of claims 1 to 7, wherein the antibody is specific for an antigen selected from integrins, interleukins, interferons, viral antigens, TNF, colony-stimulating factors and platelet-derived growth factors.

9. The method according to any one of claims 1 to 8, wherein the antibody molecule is selected from the group comprising VH, VL, VHH, Fab, modified Fab, an altered hinge Fab, Fab', $F(ab')_2$ or Fv fragment; a light chain or heavy chain monomer or dimer; a single chain antibody, e.g. a single chain Fv in which the heavy and light chain variable

domains are joined by a peptide linker, and a dual specificity antibody, such as a Fab-dAb.

10. The method according to any one of claims 1 to 9, wherein the host cell is *E. coli*.

11. The method according to claim 10, wherein the antibody molecule is expressed in the periplasm of *E. coli*.

12. The method according to any one of claims 1 to 11, wherein the method additionally comprises a filtration, centrifugation or ion exchange chromatography step, or a combination thereof.

Claims

1. A method for the manufacture of recombinant antibody molecules comprising culturing a host cell sample transformed with an expression vector encoding a recombinant antibody molecule; adding an extraction buffer to the sample; and subjecting the sample to a heat treatment step; wherein the pH of the sample is detected after addition of the extraction buffer, and optionally adjusted, to ensure that the pH of the sample is 6 to 9 prior to the heat treatment step.

2. A method according to claim 1, wherein heat treatment step is performed within the range of 30°C to 70°C.

3. A method according to claim 1 or 2, wherein the extraction buffer is selected from the group comprising NaOH, NH_4OH, Sulphuric acid, EDTA, Tris buffer and combinations thereof.

4. A method according to any one of claims 1 to 3, wherein the antibody is selective to an antigen selected from integrins such as β1 integrins e.g. VLA-4, E-selectin, P selectin or L-selectin, CD2, CD3, CD4, CD5, CD7, CD8, CD11a, CD11b, CD18, CD19, CD20, CD23, CD25, CD33, CD38, CD40, CD40L, CD45, CDW52, CD69, CD134 (OX40), ICOS, BCMP7, CD137, CD27L, CDCP1, CSF1 or CSF1-Receptor, DPCR1, DPCR1, dudulin2, FLJ20584, FLJ40787, HEK2, KIAA0634, KIAA0659, KIAA1246, KIAA1455, LTBP2, LTK, MAL2, MRP2, nectin-like2, NKCC1, PTK7, RAIG1, TCAM1, SC6, BCMP101, BCMP84, BCMP11, DTD, carcinoembryonic antigen (CEA), human milk fat globulin (HMFG1 and 2), MHC Class I and MHC Class II antigens, KDR and VEGF.

5. A method according to any one of claims 1 to 4, wherein the antibody molecule is selected from the group comprising VH, VL, VHH, Fab, modified Fab, an altered hinge Fab, Fab', $F(ab')_2$ or Fv fragment; a light chain or heavy chain monomer or dimer; a single chain antibody, e.g. a single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker, and a dual specificity antibody, such as a Fab-dAb.

6. A method according to any one of claims 1 to 5, wherein the Ph is adjusted, to ensure that the pH of the sample is 6 to 9 prior to the heat treatment step.

7 An antibody obtained/obtainable from a method as defined in any one of claims 1 to 6.

8. Use of pH controlling means, such as a buffer, to improve antibody extraction, for example primary extraction, in particular where the control ensures the pH is in the range pH 6 to 9 immediately before and/or during an extraction step, such as a heat extraction step.