detecting pH or adjusting pH. In one embodiment the method according to the second aspect of the invention does not include a step of detecting the pH of the sample or a step of pH adjustment.

The following detailed description of the invention applies to embodiments of both the first and second aspects of the present invention.

pH adjustment agent and extraction buffer

The pH adjustment must be such that the pH is sustained/maintained in the desired range of pH 6-9 during the heat treatment step.

In one embodiment the pH is adjusted with a base such as an inorganic base for example sodium hydroxide or an organic base such as triethylamine or trimethylamine.

Any suitable agent may be used to adjust the pH of the sample. The agent may be the extraction buffer or may be added before and/or after the extraction buffer. Typical agents which may be used to adjust the pH comprises or consists of one or more of the following: NaOH, NH_4OH, Sulphuric acid, EDTA, Tris buffer. Preferably the pH of the sample is adjusted using a base such as sodium hydroxide or ammonium hydroxide.

In one embodiment the extraction buffer is a Tris(hydroxymethyl)aminomethan / Ethylenedinitrilotetraacetic acid disodium salt dehydrate (Tris / EDTA) buffer typically adjusted to a desired pH by addition of HCl. Without being bound by theory it is thought that Tris and EDTA work synergistically in releasing lipopolysaccarides (LPS) from the outer membrane of *E. coli*. EDTA removes divalent cations that stabilize LPS of the outer membrane. It is thought that Tris binds to LPS and replaces Ca^{2+} and Mg^{2+}. This results in a reduction of interactions between LPS molecules and therefore increased permeability of the outer membrane (Vaara, M. 1992. Agents That Increase the Permeability of the Outer Membrane. American Society for Microbiology 56:395-411).

Heat Treatment Step

The heat treatment step in the method of the present invention is preferably as described in detail in US 5,665,866 ~~(the contents of which are incorporated herein by reference)~~. The heat treatment step makes it possible to obtain a sample of soluble, correctly folded and assembled antibody by facilitating the removal of other antibody-related material. Antibody which is "correctly folded and assembled" is shown by the presence of a single band corresponding to the expected molecular weight for assembled heavy and light chains on non-reducing SDS PAGE. Other antibody related material will

and time consuming as more purification steps may be required and/or larger quantities of chromatography materials will be needed to achieve the required purity. Substantial release of host cell DNA increases sample viscosity causing filtration and centrifugation difficulties which is a major cause of protein loss during clarification. A lysed sample (*ie.* containing host cell proteins and DNA) can also cause blockage of chromatographic materials. The non-lysing treatment step is preferably carried out as described in WO 2006/054063 ~~(the contents of which are incorporated herein by reference)~~. As described in WO 2006/054063 The non-lysing treatment step includes any treatment which does not produce lysis of a substantial proportion of the bacteria, mammalian cell, yeast, insect cell, or other organism used for recombinant antibody expression, *e.g. E. coli.* In a most preferred embodiment, the non-lysing treatment comprises pressure treatment. Alternatively, the non-lysing treatment comprises a pre-conditioning step of agitation or stirring. A "substantial proportion" includes a proportion of 80% or more of the organisms in a fermentation or culture being present in intact form, more preferably more than 85%, even more preferably more than 90%, and most preferably 95% or more being intact.

Lysis can be judged in any way known in the art, including: by viewing under a microscope, fluorescence activated cell sorting (FACS) analysis and assay of total protein versus protein in supernatant and/or in an organism (cell) pellet. In one embodiment, lysis can be judged after non-lysing treatment by comparing the total protein in a sample before and after treatment. If a treatment is causing lysis, the total protein present in the supernatant of the treated sample would increase compared to the total protein present in said untreated sample, for example measured using a Bradford assay. In a preferred embodiment, FACS analysis is performed wherein the sample is labelled with a fluorescent dye followed by non-lysing treatment and FACS analysis. Most preferably, FACS analysis is performed before treatment giving a baseline value for comparison.

Thus, non-lysing treatment can include pre-conditioning by gentle resuspension over a period of time, for example by agitation or stirring, or by manual resuspension such as by pipetting, in, *e.g.* a buffer. In one embodiment, pre-conditioning is performed for between 1 hour and 24 hours, preferably between 1 hour and 20 hours, more preferably between 2 hours and 18 hours, 4 hours and 16 hours, 6 hours and 16 hours, and most preferably for 12, 14 or 16 hours. Thus, the minimum time for pre-conditioning is 1, 2 or 4 hours and the maximum is 16, 18 or 24 hours. Pre-conditioning can be performed by

example respiratory syncytial virus or cytomegalovirus antigens, immunoglobulins, such as IgE, interferons such as interferon α, interferon β or interferon γ, tumour necrosis factor TNF (formerly known as tumour necrosis factor-α), tumor necrosis factor-β, colony stimulating factors such as G-CSF or GM-CSF, and platelet derived growth factors such as PDGF-α, and PDGF-β and where appropriate receptors thereof. Other antigens include bacterial cell surface antigens, bacterial toxins, viruses such as influenza, EBV, HepA, B and C, bioterrorism agents, radionuclides and heavy metals, and snake and spider venoms and toxins.

In one embodiment, the antibody may be used to functionally alter the activity of the antigen of interest. For example, the antibody may neutralize, antagonize or agonise the activity of said antigen, directly or indirectly.

In a preferred embodiment the antibody is an anti-TNF antibody, more preferably an anti-TNF Fab', as described in WO01/094585 ~~(the contents of which are incorporated herein by reference)~~.

Methods for the expression of recombinant proteins are well known in the art. Suitable examples of host cells for the expression of recombinant antibody molecules include bacteria such as gram positive or gram negative bacteria, *e.g. E. coli*, or yeast cells, e.g. *S. cerevisiae*, or mammalian cells, *e.g.* CHO cells and myeloma or hybridoma cell lines, *e.g.* NSO cells. Most preferably, in the methods of the invention, a recombinant antibody is produced in bacteria, *e.g. E. coli* (see Verma *et al.*, 1988, J. Immunol. Methods 216:165-181; Simmons *et al.*, 2002, J. Immunol. Methods 263:133-147).

Cells

The term "sample" used in the present invention refers to a population of cells which have been transformed with an expression vector encoding a recombinant antibody molecule. The sample may be at any suitable scale from small-scale production of antibody to large-scale manufacture of antibody for commercial purposes.

The cells used in the present invention may be for example but without limitation bacteria, especially gram-negative bacteria, yeast, mammalian or insect. Most preferably, the cells are *E. coli*. The cells may be wild-type cells or recombinant cells which have been genetically engineered. *E. coli* host cells may be naturally occurring *E. coli* strains or mutated strains capable of producing recombinant proteins. Examples of specific host *E. coli* strains include MC4100, TG1, TG2, DHB4, DH5α, DH1, BL21, K12, XL1Blue and JM109. One example is *E. coli* W3110 (ATCC 27,325) a commonly used host strain for

induction of protein expression for example where the inducer is lactose there may be a delay before induction of protein expression occurs while any pre-existing carbon source is utilized before lactose.

E. coli host cell cultures (fermentations) may be cultured in any medium that will support the growth of *E. coli* and expression of the recombinant protein. The medium may be any chemically defined medium, such as those provided in Pirt S.J. (1975) Principles of Microbe and Cell Cultivation, Blackwell Scientific Publications, with modifications where appropriate to control growth rate as described herein. An example of a suitable medium is 'SM6E' as described by Humphreys *et al.*, 2002, Protein Expression and Purification, 26:309-320.

Culturing of the *E. coli* host cells can take place in any suitable container such as a shake flask or a fermenter depending on the scale of production required. Various large scale fermenters are available with a capacity of more than 1,000 litres up to about 100,000 litres. Preferably, fermenters of 1,000 to 50,000 litres are used, more preferably 1,000 to 10,000 or 12,000 litres. Smaller scale fermenters may also be used with a capacity of between 0.5 and 1,000 litres.

Fermentation of *E. coli* may be performed in any suitable system, for example continuous, batch or fed-batch mode (Thiry & Cingolani, 2002, Trends in Biotechnology, 20:103-105) depending on the protein and the yields required. Batch mode may be used with shot additions of nutrients or inducers where required. Alternatively, a fed-batch culture may be used and the cultures grown in batch mode pre-induction at the maximum specific growth rate that can be sustained using the nutrients initially present in the fermenter and one or more nutrient feed regimes used to control the growth rate until fermentation is complete. Fed-batch mode may also be used pre-induction to control the metabolism of the *E. coli* host cells and to allow higher cell densities to be reached (Lee, 1996, Tibtech, 14:98-105).

Preferred features of each embodiment of the invention are as for each of the other embodiments *mutatis mutandis*. ~~All publications, including but not limited to patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.~~

In one aspect there is provided an antibody obtained or obtainable from said process.

CLAIMS

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

   a) culturing a host cell sample of gram-negative bacteria~~, yeast, mammalian or insect cells~~ transformed with an expression vector encoding a recombinant antibody molecule;

   b) adding an extraction buffer having a pH of 7.5 to 9 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~~ wherein the pH of the sample is detected after addition of the extraction buffer and adjusted to ensure that the pH of the sample is 7 to 9 prior to the heat treatment step and wherein the antibody molecule is selected from the group consisting of 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, a single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker, and a Fab-dAb.

2. The method according to claim 1, wherein the pH is detected before and during ~~the~~ a 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 the heat ~~the~~ treatment step.

5. The method according to claim 4, wherein pH of the sample is monitored continuously during the heat 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.~~9. The method according to any one of claims 1 to ~~9~~8, wherein the host cell is *E. coli*.

~~11.~~10. The method according to claim ~~10~~9, wherein the antibody molecule is expressed in the periplasm of *E. coli*.

~~12.~~11. The method according to any one of claims 1 to ~~11~~10, wherein the method additionally comprises a filtration, centrifugation or ion exchange chromatography step, or a combination thereof.