Patent Claims

1. A method for producing a monomeric Fc-region containing polypeptide comprising the steps

   a) ~~i) applying a solution comprising the Fc-region containing polypeptide in monomeric form and in aggregated form, wherein the aggregated form is 20 % or more as determined by size exclusion chromatography of the total Fc-region containing polypeptide in the solution, to a hydrophobic interaction chromatography material and recovering the Fc-region containing polypeptide from the flow-through, or~~

   ~~ii)~~ applying a solution comprising the Fc-region containing polypeptide in monomeric form and in aggregated form, wherein the aggregated form is 20 % or more as determined by size exclusion chromatography of the total Fc-region containing polypeptide in the solution, to a mixed-mode chromatography material and recovering the Fc-region containing polypeptide from the mixed-mode chromatography material,

   and

   b) applying the solution obtained in the previous step to a weak cation exchange chromatography material and recovering the Fc-region containing polypeptide from the flow-through of the weak cation exchange chromatography material~~therefrom~~ and thereby producing the monomeric Fc-region containing polypeptide.

2. The method according to claim 1, characterized in that the solution applied to the mixed-mode chromatography material and/or the weak cation exchange chromatography material has a pH value at least 0.2 pH units and at most 2.5 pH units above the isoelectric point of the Fc-region containing polypeptide.

3. The method according to any one of the preceding claims, characterized in that the solution applied to the mixed-mode chromatography material and/or the weak cation exchange chromatography material is a buffered solution with a buffer concentration of from about 1 mmol/l to about 150 mmol/l.

~~4. The method according to any one of the preceding claims, characterized in that the recovering from the weak cation exchange chromatography material is from the eluate of the weak cation exchange chromatography material or from the flow-through of the weak cation exchange chromatography material.~~

~~5~~4. The method according to any one of the preceding claims, characterized in that the applying the solution to a hydrophobic interaction chromatography material comprises the following steps

- applying to the hydrophobic interaction chromatography material a solution comprising Tris buffer with a pH value of about 7.0, of from about 0.5 mol/l to about 1.5 mol/l sodium chloride and of from about 10 % to about 15 % (v/v) 2-propanol,
- adjusting the solution comprising the Fc-region containing polypeptide prior to the applying to the chromatography material to a pH value of about 7.0, a sodium chloride concentration of from about 0.5 mol/l to about 1.5 mol/l and a 2-propanol content of from about 10 % to about 15 % (v/v),
- applying the solution comprising the Fc-region containing polypeptide to the hydrophobic interaction chromatography material.

~~6~~5. The method according to any one of the preceding claims, characterized in that the applying to and recovering from the mixed-mode chromatography material comprises the following steps

- applying to the mixed-mode chromatography material a solution comprising a buffer with a pH value of the IEP of the Fc-region containing polypeptide plus about 0.7 pH units to about 1.3 pH units,
- adjusting the solution comprising the Fc-region containing polypeptide prior to the applying to the chromatography material to a pH value of the IEP of the Fc-region containing polypeptide plus about 0.7 pH units to about 1.3 pH units,
- applying the solution comprising the Fc-region containing polypeptide to the mixed-mode chromatography material,
- recovering the Fc-region containing polypeptide from the mixed-mode chromatography material by applying a solution comprising a phosphate buffer with a pH value of the IEP of the Fc-region containing polypeptide plus about 1.7 pH units to about 2.3 pH units.

~~7~~6. The method according to any one of the preceding claims, characterized in that the applying to the weak cation exchange chromatography material and the recovering of the Fc-region containing polypeptide comprises the following steps

- applying to the weak cation exchange chromatography material a solution comprising a buffer with a pH value of the IEP of the Fc-region containing polypeptide plus about 0.2 pH units to about 1.0 pH units,

- adjusting the solution comprising the Fc-region containing polypeptide prior to the applying to the chromatography material to a pH value of the IEP of the Fc-region containing polypeptide plus about 0.2 pH units to about 1.0 pH units,

- applying the solution comprising the Fc-region containing polypeptide to the weak cation exchange chromatography material,

- recovering the Fc-region containing polypeptide from the weak cation exchange chromatography material by applying a solution comprising sodium chloride or potassium chloride at a concentration of from about 210 mmol/l to about 240 mmol/l, or

  recovering the Fc-region containing polypeptide from the weak cation exchange chromatography material with a solution of a pH value of from about pH 6.0 to about pH 8.0.

~~8~~7. The method according to claim ~~7~~6, characterized in that in the recovering the pH value is about pH 5.0 and the sodium chloride concentration is about 230 mmol/l, or the pH value is about pH 5.2 and the sodium chloride concentration is about 210 mmol/l.

~~9~~8. The method according to any one of the preceding claims, characterized in that the applying to the weak cation exchange chromatography material and the recovering of the Fc-region containing polypeptide comprises the following steps

- applying to the weak cation exchange chromatography material a solution with a pH value of the IEP of the Fc-region containing polypeptide plus about 0.2 pH units to about 1.2 pH units,

- adjusting the solution comprising the Fc-region containing polypeptide prior to the applying to the chromatography material to a pH value of the IEP of the Fc-region containing polypeptide plus about 0.2 pH units to about 1.2 pH units,

- applying the solution to a weak cation exchange chromatography material,

- recovering the Fc-region containing polypeptide from the flow-through.

~~10~~9. The method according to claim ~~9~~8, characterized in that the adjusting is to an acetate buffer of about pH 5.1 and of from about 100 mol/l to about 120 mmol/l, or to an acetate buffer of about pH 5.5 and of about 40 mmol/l to about 55 mmol/l, or to an acetate buffer of about pH 6.0 and of about 10 mmol/l.

~~11~~10. The method according to any one of claims 3 to ~~9~~8, characterized in that the buffer is selected from citrate buffer, acetate buffer and MES buffer.

~~12~~11. The method according to any one of claims ~~7~~6 to ~~11~~10, characterized in that the recovering is by an isocratic elution.

~~13~~12. The method according to any one of claims ~~6~~5 to ~~12~~11, characterized in that the buffer has a conductivity of about 1.9 mS/cm.

~~14~~13. The method according to any one of claims ~~9~~8 and ~~10~~9 to ~~13~~12, characterized in that the buffer in step ii) is an acetate buffer of about pH 5.3 and of about 50 mmol/l to about 75 mmol/l, or an acetate buffer of about pH 5.5 and of about 7.5 mmol/l to about 15 mmol/l.

~~15~~14. The method according to any one of claims ~~7~~6, ~~8~~7, and ~~11~~10 to ~~14~~13, characterized in that the recovering is by adding 10 % (v/v) of an acetate buffer of a pH value of from about pH 6.0 to about pH 6.5 or the recovering is by adding 60 % (v/v) of a MES buffer of a pH value of about pH 8.0.