

CLAIMS

- 1) A method for manufacturing an antibody or antigen-binding fragment comprising:
 - a) culturing a host cell under conditions such that they express the antibody or antigen-binding fragment thereof,
 - b) purifying said antibody or antigen-binding fragment thereof from a mixture containing the host cells and other contaminants, wherein said purification comprises at least one chromatography step and wherein a reducing agent is added to said mixture and the antibody or antigen-binding fragment thereof is maintained in the presence of said reducing agent from the first chromatography step until the last chromatography step,
 - c) adding polyethylene glycol (PEG) to the antibody or antigen-binding fragment recovered from step b), and
 - d) recovering said antibody or antigen-binding fragment thereof coupled to a PEG molecule via covalent binding to a cysteine residue on the antibody or antigen-binding fragment thereof.
- 2) A method according to claim 1 wherein the reducing agent is selected from glutathione, β -mercaptoethanol, β -mercaptoethylamine, dithiothreitol, Tris(2-carboxyethyl)phosphine, cysteine, and combinations thereof.
- 3) The method according to claims 1 or 2, wherein the reducing agent is 0.1 mM to 100 mM glutathione.
- 4) The method according to claim 3, wherein the reducing agent is 0.5 mM to 2 mM glutathione.
- 5) The method according to any preceding claim, wherein the reducing agent is removed from the recovered antibody or antigen-binding fragment in step b).
- 6) The method according to any preceding claim, wherein said antigen binding fragment is a Fab'.
- 7) The method according to any preceding claim, wherein said cysteine in said antibody or antigen binding fragment is in the antibody hinge.
- 8) The method according to any preceding claim, wherein said PEG molecule is 40,000 PEG-maleimide.
- 9) The method according to any preceding claim wherein the purification of the antibody or antibody-binding fragment thereof of step b) comprises a first chromatography step that is a cation exchange chromatography from where a first eluate containing the antibody or antibody-binding fragment thereof is eluted, and a second chromatography step that is an anion exchange chromatography to produce a flow-through containing the antibody or antibody-binding fragment thereof.