

CLAIMS

- 1) A method for manufacturing an antibody or antigen-binding fragment thereof comprising:
 - a) culturing an *E. coli* host cell under conditions such that they express the antibody or antigen binding fragment thereof in the periplasm,
 - b) adding an extraction buffer to the host cells and subjecting the host cells to a heat treatment step,
 - c) purifying said antibody or antigen binding region 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,
 - d) adding polyethylene glycol (PEG) to the antibody or antigen binding region thereof,
 - e) coupling the protein to the PEG via a covalent binding to a cysteine residue present in the hinge region of the antibody or antigen binding fragment thereof and
 - f) recovering said antibody or antigen binding fragment thereof coupled to PEG,wherein the reducing agent is 0.5 mM to 2 mM glutathione.
- 2) The method according to any preceding claim, wherein the reducing agent is removed from the recovered antibody or antigen-binding fragment in step c).
- 3) The method according to any preceding claim, wherein said antigen binding fragment is a Fab'.
- 4) The method according to any preceding claim, wherein said PEG molecule is 40,000 PEG-maleimide.
- 5) The method according to any preceding claim wherein the purification of the antibody or antibody-binding fragment thereof of step c) 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.