

WHAT IS CLAIMED IS:

1. A method for determining the total carbonylation level on a polypeptide wherein the method comprises the steps of
 - a) Contacting the polypeptide in a solution with a hydrazide dye under conditions such that the hydrazide dye can react with the carbonyl groups present on the amino acid residues of the polypeptide to form a polypeptide-dye-complex,
 - b) Removing unbound hydrazide dye from the resultant solution from step a),
 - c) Determining in the resultant solution from step b) the concentration of the hydrazide dye bound in the polypeptide-dye-complex and the concentration of the polypeptide bound in the polypeptide-dye-complex, and
 - d) Determining the total carbonylation level of the polypeptide based on the ratio of the concentration of the hydrazide dye bound in the polypeptide-dye-complex to the concentration of the polypeptide bound in the polypeptide-dye-complex.
2. The method according to claim 1, wherein the hydrazide dye is LY-CH.
3. The method according to claim 1, wherein step a) is performed in the presence of alkali metal ions.
4. The method according to claim 3, wherein the alkali metal ions are lithium ions.
5. The method according to claim 1, wherein the amino acid residues of the polypeptide are selected from the group consisting of arginine, lysine, proline and threonine.
6. The method according to claim 1, wherein the polypeptide is an antibody.
7. The method according to claim 6, wherein the antibody is a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric

antibody, a multi-specific antibody, an antibody fragment, an antibody drug conjugate, a THIOMABTM or a THIOMABTM drug conjugate.

8. The method according to claim 1, wherein the polypeptide and the hydrazide dye is contacted in step a) in a final molar ratio of 6,000 – 10,000.
9. The method according to claim 1, wherein step a) is performed in the presence of a non-ionic surfactant.
10. The method according to claim 9, wherein the non-ionic surfactant is reduced Triton X-100.
11. The method according to claim 1, wherein step a) is performed at a temperature of 35°C – 39°C.
12. The method according to claim 1, wherein step a) is performed for 10 – 20 h.
13. The method according to claim 1, wherein step b) is performed by a method selected from the group consisting of filtration, gel filtration and dialysis.
14. The method according to claim 1, wherein step b) is performed by filtration.
15. The method according to claim 14, wherein filtration is carried out in the presence of 100 – 300 mM potassium phosphate and in the presence of 200 – 300 mM potassium chloride.
16. A kit for carrying out the method according to claim 1, wherein the kit comprises a hydrazide dye and a buffer.
17. The kit according to claim 16, wherein the hydrazide dye is LY-CH.

18. The kit according to claim 16, wherein the buffer is lithium MES buffer.
19. The kit according to claim 16, wherein the kit further comprises a non-ionic surfactant, a standard for the polypeptide and a standard for the hydrazide dye.
20. The kit according to claim 19, wherein the non-ionic surfactant is reduced Triton X-100.