CLAIMS

1. A composition, combination, or kit comprising a polypeptide having endoprotease activity specific for O-glycosylated proteins ~~which~~ , wherein the polypeptide comprises:

(a) an amino acid sequence of SEQ ID NO: 1;

(b) an amino acid sequence which is at least 85% identical to the amino acid sequence of SEQ ID NO: 1 or

(c) an amino acid sequence which is a fragment of the sequence of SEQ ID NO: 1 or a fragment of an amino acid sequence which is 85% identical to the amino acid sequence of SEQ ID NO: 1,

and wherein the polypeptide is provided together with a sialidase Am1757 or a mixture of Am1757 and Am0707, wherein Am1757 is a polypeptide of any one of SEQ ID NOs: 9 to 11 and Am0707 is a polypeptide of any one of SEQ ID NOs: 12 to 14.

2. The composition, combination, or kit ~~polypeptide~~ according to claim 1, wherein the polypeptide comprises an amino acid sequence which is at least 85% identical to the amino acid sequence of SEQ ID NO: 1, or the fragment thereof, comprises the motif HEbbH, wherein b is an uncharged amino acid selected from A, C, F, G, I, L, M, N, P, Q, S, T, V or W.

3. The composition, combination, or kit ~~polypeptide~~ according to claim 2, wherein said motif is present in said polypeptide at positions corresponding to positions 181 to 185 of SEQ ID NO: 1.

4. The composition, combination, or kit ~~polypeptide~~ according to claim 2 or claim 3, wherein said motif comprises the sequence HEIGH or HELGH.

5. The composition, combination, or kit ~~polypeptide~~ according to any one of the preceding claims ~~which~~ wherein the polypeptide comprises the motif abxHEbbHbc, wherein:

(a) a is amino acid V, T or G;

(b) b is an uncharged amino acid selected from A, C, F, G, I, L, M, N, P, Q, S, T, V or W;

(c) x is any amino acid; and

(d) c is a hydrophobic amino acid selected from A, C, F, I, L, M, P, V, W or Y.

6. The composition, combination, or kit ~~polypeptide~~ according to claim 5, wherein said motif comprises the sequence GMAHELGHGL or GVAHELGHNF.

7. The composition, combination, or kit ~~polypeptide~~ according to any one of the preceding claims, ~~which~~ wherein the polypeptide includes an additional methionine at the N terminus and/or a His tag at the C terminus, which tag may be joined to the C terminus by a linker.

8. The composition, combination, or kit ~~polypeptide~~ according to claim 7, wherein said polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 2.

9. The composition, combination, or kit ~~polypeptide~~ according to any one of claims 1 to 6, wherein the polypeptide is provided in solution, lyophilised, or immobilised.

10. The composition, combination, or kit ~~polypeptide~~ according to claim 9, wherein Am1757 is a polypeptide consisting of SEQ ID NO: 11 and/or wherein Am0707 is a polypeptide consisting of SEQ ID NO: 14.

11. A method of hydrolysing an O-glycoprotein, wherein the method comprises contacting a sample comprising the protein with a ~~polypeptide~~ composition or combination according to any one of claims 1 to 10 and thereby hydrolysing the O-glycoprotein.

12. The method according to claim 11, further comprising the detection or analysis of the hydrolysis products.

13. A method for assessing the glycosylation status of a protein, comprising contacting a sample comprising the protein with a ~~polypeptide~~ composition or combination according to any one of claims 1 to 10 and detecting and/or analysing the products produced.

14. The method according to claim 13, wherein the presence or absence of cleavage products is used to determine the presence or absence of an O-glycoprotein in the sample, and/or wherein said analysis is conducted to identify the type of a O-glycan chain and/or its position of attachment to an O-glycoprotein.

15. The method according to any one of claims 11 to 14, wherein the analysis or detection is carried out by affinity chromatography, SDS-PAGE, HPLC or mass spectrometry.

~~16. The method according to any one of claims 11 to 15, wherein the sample is incubated with a sialidase prior to or at the same time as contacting the protein or sample with the polypeptide of claims 1 to 10.~~

~~17. The method according to claim 16, wherein said sialidase is Am1757 or a mixture of Am1757 and Am0707, and wherein Am1757 is a polypeptide of any one of SEQ ID NOs: 9 to 11 and Am0707 is a polypeptide of any one of SEQ ID NOs: 12 to 14.~~

~~18. The method according to claim 16 or claim 17, wherein said sialidase is Am1757 or a mixture of Am1757 and Am0707, and wherein Am1757 is a polypeptide consisting of SEQ ID NO: 11 and/or wherein Am0707 is a polypeptide consisting of SEQ ID NO: 14.~~

~~19~~16. A polypeptide which is capable of binding to an O-glycan or O-glycoprotein and which lacks or has reduced endoprotease activity specific for O-glycosylated proteins comprising an amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 20.

~~20~~17. The polypeptide according to claim ~~19~~ 16, which includes an additional methionine at the N terminus and/or a His tag at the C terminus, which tag may be joined to the C terminus by a linker.

~~21~~18. The polypeptide according to claim ~~20~~ 17, wherein said polypeptide comprises or consists of the amino acid sequence of SEQ ID NO: 6 or 21.

~~22~~19. The polypeptide according to any one of claims ~~19~~ 16 to ~~21~~ 18, wherein the polypeptide is provided in solution, lyophilised, or immobilised.

~~23~~20. A composition, combination, or kit comprising the ~~The~~ polypeptide according to claim ~~22~~ 19 and one or more~~, wherein the polypeptide is provided together with a~~ sialidase.

~~24~~21. The composition, combination, or kit ~~polypeptide~~ according to claim ~~23~~ 20, wherein the one or more sialidase is Am1757 or a mixture of Am1757 and Am0707, and

wherein Am1757 is a polypeptide of any one of SEQ ID NOs: 9 to 11 and Am0707 is a polypeptide of any one of SEQ ID NOs: 12 to 14.

~~25~~22. A method of binding to an O-glycan, O-glycopeptide and/or O-glycoprotein wherein the method comprises contacting a sample comprising the O-glycan, O-glycopeptide and/or O-glycoprotein with ~~a~~ the polypeptide according to any one of claims ~~19~~ 16 to 19, or a composition or combination according claim 20 or claim 21.

~~26~~23. The method according to claim ~~25~~ 22, further comprising determining whether or not an O-glycan, O-glycopeptide and/or O-glycoprotein has been bound by the polypeptide and/or separating the O-glycan and any linked glycoprotein, the O-glycopeptide or O-glycoprotein from the resulting mixture

~~27~~24. The method according to claim ~~25~~ 22 or claim ~~26~~ 23, wherein the method is for the purpose of isolating an O-glycan or linked glycoprotein, O-glycopeptide or O-glycoprotein from the sample.

~~28~~25. A method for assessing the glycosylation status of a protein, comprising contacting a sample comprising the protein with ~~a~~ the polypeptide according to any one of claims ~~19~~ 16 to 19, or a composition or combination according claim 20 or claim 21 and determining whether or not the protein is bound by the ~~said~~ polypeptide.

~~29~~26. A method for detecting O-glycopeptides and/or O-glycoproteins in a sample, wherein the method comprises:

(a) contacting said sample with a polypeptide according to any one of claims ~~19~~ 16 to 19, or a composition or combination according claim 20 or claim 21 to thereby allow formation of complex between an O-glycopeptide and/or O-glycoprotein and the ~~said~~ polypeptide; and

(b) determining whether the separated polypeptide is bound to an O-linked glycoprotein or glycopeptide**,** thereby determining the presence or absence of O-linked glycopeptides or glycoproteins in the sample.

~~30~~27. The method according to claim ~~29~~ 26, further comprising a step of separating said polypeptide from the contacted sample between steps (a) and (b).

~~31~~28. The method according to any one of claims ~~25~~ 22 to ~~30~~ 27, wherein said determining and/or separating is carried out by affinity chromatography, SDS-PAGE, HPLC, lectin blotting, ELISA or mass spectrometry.

~~32~~29. The method according to any one of claims ~~25~~ 22 to ~~31~~ 28 which additionally comprises a step of eluting bound material from the polypeptide of any one of claims ~~19~~ 16 to 19, or the polypeptide comprised within the composition or combination according claim 20 or claim 21 with a buffer comprising:

(a) High molar concentration Urea;

(b) High concentration detergent; or

(c) A polypeptide comprising:

(i) an amino acid sequence of SEQ ID NO: 1;

(ii) an amino acid sequence which is at least 85% identical to the amino acid sequence of SEQ ID NO: 1, or

(iii) an amino acid sequence which is a fragment of the sequence of SEQ ID NO: 1 or a fragment of an amino acid sequence which is 85% identical to the amino acid sequence of SEQ ID NO: 1.~~,~~

~~according to any one of claims 1 to 8~~.