CLAIMS

1. A composition, combination, or kit comprising a polypeptide having endoprotease activity specific for O-glycosylated proteins, 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 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 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 according to claim 2 or claim 3, wherein said motif comprises the sequence HEIGH or HELGH.

5. The composition, combination, or kit according to any one of the preceding claims 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 according to claim 5, wherein said motif comprises the sequence GMAHELGHGL or GVAHELGHNF.

7. The composition, combination, or kit according to any one of the preceding claims, 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 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 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 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 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 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. 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.

17. The polypeptide according to claim 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.

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

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

20. A composition, combination, or kit comprising the polypeptide according to claim 19 and one or more sialidase.

21. The composition, combination, or kit according to claim 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.

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 the polypeptide according to any one of claims 16 to 19, or a composition or combination according claim 20 or claim 21.

23. The method according to claim 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

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

25. A method for assessing the glycosylation status of a protein, comprising contacting a sample comprising the protein with the polypeptide according to any one of claims 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 polypeptide.

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 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 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.

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

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

29. The method according to any one of claims 22 to 28 which additionally comprises a step of eluting bound material from the polypeptide of any one of claims 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.

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