CLAIMS

1. An isolated polypeptide comprising

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

(b) a variant thereof having at least ~~50~~95% identity to the amino acid sequence of SEQ ID NO: 1 over at least 810 contiguous amino acids of SEQ ID NO: 1 and having the ~~endogycosidase~~endoglycosidase activity of a polypeptide consisting of the amino acid sequence of SEQ ID NO: 1.~~; or~~

~~(c) a fragment of either thereof having endoglycosidase activity.~~

2. A polypeptide according to claim 1 which consists of the amino acid sequence of SEQ ID NO: 1.

~~2~~3. An isolated polypeptide capable of binding to IgG and which does not have endoglycosidase activity comprising

(a) the amino acid sequence of SEQ ID NO: 2;

(b) a variant thereof having at least ~~50~~95% identity to the amino acid sequence of SEQ ID NO: 1 over at least 810 contiguous amino acids of SEQ ID NO: 1, in which the amino acid equivalent to glutamic acid at position 186 is substituted~~; or~~.

~~(c) a fragment of either thereof.~~

~~3~~4. A polynucleotide comprising a sequence which encodes a polypeptide as defined in claim 1~~ or claim~~, 2 or 3.

~~4~~5. A polynucleotide according to claim ~~3~~4 which comprises

(i) SEQ ID NO: 3 or a complementary sequence thereto;

(ii) a sequence which hybridises under stringent conditions to the sequence defined in (i);

(iii) a sequence which is degenerate as a result of the genetic code to a sequence as defined in (i) or (ii);

(iv) a sequence having at least 60% identity to a sequence as defined in (i), (ii) or (iii);

(v) a fragment of any of the sequences (i), (ii), (iii) or (iv), and which encodes a polypeptide having the endoglycosidase activity of the polypeptide of SEQ ID NO: 1;-or

(vi) a variant of any of the sequences of (i), (ii), (iii), (iv) or (v), which comprises a substitution at the encoded amino acid for glutamic acid at position 186 or an equivalent position thereof, which encodes a polypeptide which does not have endoglycosidase activity, but is capable of binding to IgG.

~~5~~6. An expression vector, comprising a polynucleotide as defined in claim ~~3~~4 or 45.

~~6~~7. A method for hydrolysing glycan of a glycoprotein comprising incubating the glycoprotein with a polypeptide as defined in claim 1 or 2.

~~7~~8. A method for assessing the glycosylation status of a glycoprotein comprising incubating a glycoprotein with a polypeptide according to claim 1 or 2, and analysing the products produced.

~~8~~9. The method according to claim ~~7~~8 ~~wherein the method~~which comprises:

(a) contacting the glycoprotein with ~~a~~ the said polypeptide ~~according to claim 1~~ to hydrolyse glycan of the glycoprotein;

(b) separating the glycan from the deglycosylated protein;

(c) analysing the glycan and/or deglycosylated protein so produced.

~~9~~10. The method according to any one of claims ~~6, or 7~~ 7 to 9 wherein the glycoprotein comprises an IgG antibody or a monoclonal IgG antibody.

~~10~~11. A method for isolating IgG from an IgG-containing sample, which method comprises:

(a) contacting said IgG-containing sample with an EndoS49 polypeptide which lacks IgG endoglycosidase activity, to thereby allow formation of a IgG-EndoS49 polypeptide complex;

(b) separating said EndoS49 from the contacted sample; and

thereby obtaining isolated IgG.

~~11~~12. A method for determining the presence or absence of IgG in a sample, which method comprises:

(a) contacting said sample with an EndoS49 polypeptide which lacks IgG endoglycosidase activity, to thereby allow formation of a IgG-EndoS49 polypeptide complex;

(b) optionally separating said EndoS49 from the contacted sample; and

(c) determining whether the separated EndoS49 is bound to IgG; and

thereby determining the presence or absence of IgG in the sample.

~~12~~13. Method according to claim ~~10~~ 11 or ~~11~~12, wherein said EndoS49 polypeptide comprises:

(a) the amino acid sequence of SEQ ID NO: 2;

(b) a variant thereof having at least ~~50~~95% identity to the amino acid sequence of SEQ ID NO: 2 over at least 820 contiguous amino acids of SEQ ID NO: 2 and having IgG-binding activity.~~; or~~

~~a fragment of either thereof having IgG-binding activity.~~

~~13~~14. Method according to any one of claims ~~10~~ 11 to ~~12~~13, wherein the isolated or detected IgG is in its native, functionally active form.

~~14~~15. Method according to any one of claims ~~10~~ 11 to ~~13~~14, wherein the isolated or detected IgG comprises at least one, preferably two, three or all four of the IgG subclasses, IgG_1, IgG_2, IgG_3 and IgG_4.

~~15~~16. Method according to any one of claims ~~10~~ 11 to ~~14~~ 15 wherein step(a) additionally comprises contacting the sample with a population of magnetic nanoparticles capable of binding to the EndoS49 polypeptide, and wherein step (b) comprises carrying out magnetic field separation on the contacted sample.

~~16~~17. A method of assessing the glycosylation state or functional quality of an IgG containing sample, which method comprises taking a first and a second sub-sample of the IgG-containing sample, and wherein steps (a) and (b) according to any one of claims ~~10~~ 11 or ~~12~~ 13 to ~~14~~ 15 are applied to the first sub-sample, and steps (a) and (b) according to any one of claims ~~10~~ 11 or ~~12~~ 13 to ~~14~~ 15 are applied to the second sub-sample except the EndoS49 polypeptide is substituted with an alternative IgG-binding reagent which is capabale of binding unglycosylated and/or denatured, inactive IgG, and further comprising:

(a) quantifying the amount of IgG bound to the EndoS49 polypeptide in the first sub-sample, and the amount of IgG bound to the alternative IgG-binding reagent in the second sub-sample; and

(b) comparing both of the amounts of bound IgG determined in (c);

and thereby assessing the glycosylation state or functional quality of an IgG containing sample.

~~17~~18. Method according to claim ~~16~~ 17 wherein the alternative IgG-binding reagent comprises Protein G and/or Protein A.

~~18~~19. A method for isolating Fab fragments from IgG-containing samples, which method comprises:

(a) contacting said IgG-containing sample with IdeS, and an EndoS49 polypeptide;

(b) separating said IdeS and said EndoS49 polypeptide from the contacted sample; and

thereby isolating Fab fragments.

~~19~~20. Method according to claims ~~18~~ 19 wherein step(a) additionally comprises contacting the sample with a population of magnetic nanoparticles capable of binding to the IdeS polypeptide and the EndoS49 polypeptide, and wherein step (b) comprises carrying out magnetic field separation on the contacted sample, and/or wherein the EndoS49 polypeptide is an EndoS49 polypeptide as defined in claim ~~12.~~13.

~~20~~21. A method for isolating Fc fragments from IgG-containing samples, which method comprises:

(a) contacting said IgG-containing sample with IdeS;

(b) separating IdeS from the mixture obtained in step (a), thereby isolating Fab and Fc fragments;

(c) contacting said Fab and Fc fragments with an EndoS49 polypeptide to thereby allow formation of a Fc fragment-EndoS49 polypeptide complex;

(d) separating the Fc fragment-EndoS49 polypeptide complexes from the mixture obtained in step (c); and

(e) isolating Fc fragments from the Fc fragment-EndoS49 polypeptide complexes obtained in step (d).

~~21~~22. Method according to claim ~~20~~ 21 wherein step (a) additionally comprises contacting the sample with a population of magnetic nanoparticles capable of binding to IdeS, step (c) additionally comprises contacting the isolated Fab and Fc fragments obtained in step (b) with a population of magnetic nanoparticles capable of binding to the EndoS49 polypeptide, and wherein steps (b) and (d) comprise carrying out

magnetic field separation on the contacted sample or isolated Fab and Fc fragments, respectively and/or wherein the EndoS49 polypeptide is an EndoS49 polypeptide as defined in claim ~~12~~13.

~~22~~23. Method according to any one of claims ~~18~~ 19 to ~~21~~22, which method comprises prior to step (a):

(i) contacting said IgG-containing sample with an EndoS49 polypeptide as defined in claim ~~12~~13, to thereby allow formation of a IgG-EndoS49 polypeptide complex;

(ii) separating said IgG-EndoS49 polypeptide complex from the contacted sample;

(iii) eluting IgG from the IgG-EndoS49 polypeptide complex thereby obtaining an IgG-containing sample;

and wherein steps (a) and (b) are carried out the IgG-containing sample obtained in step (iii).

~~23~~24. A kit for isolating IgG from an IgG-containing sample, comprising:

(a) an EndoS49 polypeptide as defined in claim ~~12~~13; and optionally

(b) means for separating said EndoS49 polypeptide from a sample.

~~24~~25. A kit for determining the presence or absence of IgG in a sample, comprising:

(a) an EndoS49 polypeptide as defined in claim ~~12~~13; and optionally

(b) means for separating said EndoS49 polypeptide from a sample.

~~25~~26. A kit for assessing the glycosylation state or functional quality of an IgG containing sample, comprising:

(a) an EndoS49 polypeptide as defined in claim ~~12~~13;

(b) an alternative IgG-binding reagent which is capable of binding denatured and/or deglycosylated IgG;

(c) means for separating said EndoS49 polypeptide and said alternative IgG-binding reagent from a sample.

~~26~~27. Kit according to claim ~~25~~ 26 wherein the alternative IgG-binding reagent comprises Protein G and/or Protein A and/or Protein A/G.

~~27~~28. Kit for isolating Fab or Fc fragments of IgG comprising:

(a) IdeS;

(b) an EndoS49 polypeptide; and

(c) means for separating said IdeS and said EndoS49 polypeptide from a sample.

~~28~~29. Kit according to claim ~~27~~ 28 wherein the EndoS49 polypeptide is said EndoS49 polypeptide as defined in claim ~~12~~13.

~~29~~30. Kit according to any one of claims ~~23~~ 24 to ~~28~~29, wherein the means for separating said EndoS49 polypeptide, said alternative IgG-binding reagent, or said IdeS from a sample are populations of magnetic nanoparticles, wherein each population is capable of binding to at least one of the indicated polypeptides/reagents/proteins.~~, and optionally wherein the kit additionally comprises instructions to perform magnetic field separation on the sample.~~