Patent Claims

1. Method for producing an immunoglobulin or polypeptide comprising at least the CH2 domain and the CH3 domain of a full length immunoglobulin heavy chain with defined glycostructure comprising the following steps:

   - providing an affinity chromatography column eluate containing the immunoglobulin or immunoglobulin fragment,
   - incubating the affinity chromatography column eluate with an α–galactosidase of plant origin, which cleaves off the (α1,3)glycosidically bound galactose residue at the non-reducing end of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment, wherein said α–galactosidase is (α1,3)galactosidase (EC 3.2.1.22) from green coffee beans,
   - applying the incubated affinity chromatography eluate to a protein A chromatography material and recovering the immunoglobulin or immunoglobulin fragment from the protein A chromatography material and thereby producing an immunoglobulin or immunoglobulin fragment with defined glycostructure.

~~2. Method according to claim 1, characterized in that the enzyme, which cleaves off the monosaccharide residue at the non-reducing end of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment, is (α1,3)galactosidase (EC 3.2.1.22) from green coffee beans.~~

~~3~~2. Method according to ~~any one of~~ claims 1 ~~or 2~~, characterized in that the incubating the affinity chromatography column eluate with said (α1,3)galactosidase (EC 3.2.1.22) from green coffee beans~~an enzyme, which cleaves off the monosaccharide residue at the non-reducing end of the glycostructure in the CH2 domain of the immunoglobulin or immunoglobulin fragment~~, comprises the following steps:

   - incubating the affinity chromatography column eluate with ~~an (α1,3)glycosidase~~said (α1,3)galactosidase (EC 3.2.1.22) from green coffee beans,
   - taking a sample from the incubation mixture,
   - either
     - applying the sample to protein A coated sepharose beads and afterwards washing the beads,

- recovering the immunoglobulin or immunoglobulin fragment from the protein A sepharose beads,
- adjusting the buffer conditions for glycosidase/sialidase digest, and
- incubating the immunoglobulin or immunoglobulin fragments with a glycosidase/sialidase to cleave of all N-Glycans,

or

- applying the sample to protein A coated magnetic beads and afterwards washing the beads,
- incubating the beads with a glycosidase and recovering the cleaved off oligosaccharides, and
- purifying the cleaved off oligosaccharides with a cation exchange chromatography,

- determining the kind and amount of the galactose ~~monosaccharide~~ residue at the non-reducing end of the glycostructure in the cleaved off oligosaccharides by mass spectrometry,
- continuing the incubating until all galactose ~~monosaccharide~~ residue at the non-reducing end of the glycostructure, which can be cleaved off by the enzyme, have been cleaved off.

43. Method according to any one of claims 1 or 2~~the preceding claims~~ further comprising the following steps as first steps:

- providing a cell comprising a nucleic acid encoding the immunoglobulin or immunoglobulin fragment,
- cultivating the cell,
- recovering the immunoglobulin or immunoglobulin fragment from the cell or the cultivation medium,
- applying the immunoglobulin or immunoglobulin fragment to a protein A chromatography material and recovering the immunoglobulin from the protein A chromatography material.

~~54~~. Method according to any one of the preceding claims further comprising the following step as final step:

- purifying the produced immunoglobulin or immunoglobulin fragment with a defined glycostructure with one to three chromatography steps.

5 ~~6~~5. Method according to any one of claims ~~4~~3 to ~~5~~4, characterized in that the cell is a mammalian cell.

~~7~~6. Method according to claim ~~6~~5, characterized in that the mammalian cell is a hamster cell, murine cell, a rabbit cell, a sheep cell, or a hybridoma cell
10 thereof.

~~8~~7. Method according to any one of claims ~~6~~5 or ~~7~~6, characterized in that the cell is a CHO cell, a NS0 cell, a BHK cell or a SP2/0 cell.

15 ~~9~~8. Method according to any one of the preceding claims characterized in that the immunoglobulin or immunoglobulin fragment comprises an amino acid sequence corresponding to amino acid residues 18 to 364 of SEQ ID NO:02.

~~10~~9. Use of an (α1,3)galactosidase from green coffee beans (EC 3.2.1.22) for
20 cleaving off the terminal (α1,3)glycosidically bound galactose residues from the glycostructures attached to the amino acid asparagine of the CH2 domain of a recombinantly produced immunoglobulin or immunoglobulin fragment.