

a1. providing a cell culture supernatant containing the antibody;

b1. processing the cell culture supernatant through an affinity chromatography resin to obtain an affinity chromatography pool containing the antibody;

c1. inactivating viruses in the affinity chromatography pool to obtain a virus inactivated solution;

d1. treating the virus inactivated solution with an activated carbon material to obtain an activated carbon material pool; and

e1. processing the activated carbon material pool through a cation exchange material to obtain a cation exchange material pool.

[2] ~~The~~A method for purifying an antibody from a composition containing one or more impurities~~according to [1]~~, comprising the steps of:

a2. providing a cell culture supernatant containing the antibody;

b2. processing the cell culture supernatant through an affinity chromatography resin to obtain an affinity chromatography pool containing the antibody;

c2. inactivating viruses in the affinity chromatography pool to obtain a virus inactivated solution;

d2. processing the virus inactivated solution through a depth filter to obtain a depth filtration pool;

Claims

[Claim 1]

A method for purifying an antibody from a composition containing one or more impurities, comprising the steps of:

a1. providing a cell culture supernatant containing the antibody;

b1. processing the cell culture supernatant through a Protein A~~an~~ affinity chromatography resin to obtain an affinity chromatography pool containing the antibody;

c1. inactivating viruses in the affinity chromatography pool to obtain a virus inactivated solution;

d1. treating the virus inactivated solution with an activated carbon material to obtain an activated carbon material pool; and

e1. processing the activated carbon material pool through a cation exchange material in flow-through (F/T) mode to obtain a cation exchange material pool,

wherein the solution to be loaded on the activated carbon material, and an equilibration buffer and a wash buffer for the activated carbon material each have a pH of 4.5 to 7.5 or an electrical conductivity of 2 to 10 mS/cm, or both.

[Claim 2]

~~The~~A method for purifying an antibody from a composition containing one or more impurities~~—according to claim 1~~, comprising the steps of:

a2. providing a cell culture supernatant containing the antibody;

b2. processing the cell culture supernatant through a Protein A~~an~~ affinity chromatography resin to obtain an affinity chromatography pool containing the antibody;

c2. inactivating viruses in the affinity chromatography pool to obtain a virus inactivated solution;

d2. processing the virus inactivated solution through a depth filter to obtain a depth filtration pool;

e2. treating the depth filtration pool with an activated carbon material to obtain an activated carbon material pool; and

f2. processing the activated carbon material pool through a cation exchange material in flow-through (F/T) mode to obtain a cation exchange material pool,

wherein the solution to be loaded on the activated carbon material, and an equilibration buffer and a wash buffer for the activated carbon material each have a pH of 4.5 to 7.5 or an electrical conductivity of 2 to 10 mS/cm, or both.

[Claim 3]

The method according to claim 1 or 2, wherein the affinity chromatography resin is a protein A resin.

~~[Claim 4]~~

~~— The method according to claim 3, wherein the protein-A resin is KanCapA, KanCapA3G, MabSelect SuRe, MabSelect SuRe pcc, MabSelect Prisma or Amsphere A3.~~

[Claim 45]

The method according to any one of claims 1 to 34, wherein the virus inactivation is a treatment with at least one selected from the group consisting of an acid, a surfactant, a chemical substance, a nucleic acid cross-linking agent, ultraviolet rays, gamma rays and heat.

[Claim 56]

The method according to claim 45, wherein the virus inactivation comprises lowering pH of the affinity chromatography pool to 3 to 4.

[Claim 67]

The method according to claim 56, wherein the affinity chromatography pool is incubated for 60 to 120 minutes during the virus inactivation, and/or—

~~[Claim 8]~~

~~— The method according to claim 6 or 7, wherein the affinity chromatography pool is incubated at 15 to 30°C during the virus inactivation.~~

[Claim 79]

The method according to any one of claims 1 to 68, wherein the solution to be loaded on the activated carbon material, and an equilibration buffer and a wash buffer

for the activated carbon material each have a pH of 4.5 to 7.5, for example.

~~[Claim 10]~~

~~— The method according to claim 9, wherein the solution to be loaded on the activated carbon material, and the equilibration buffer and the wash buffer for the activated carbon material each have a pH of 4.5 to 5.5.~~

[Claim 811]

The method according to any one of claims 1 to 710, wherein the solution to be loaded on the activated carbon material, and an equilibration buffer and a wash buffer for the activated carbon material each have an electrical conductivity of 2 to 10 mS/cm, for example.

~~[Claim 12]~~

~~— The method according to claim 11, wherein the solution to be loaded on the activated carbon material, and the equilibration buffer and the wash buffer for the activated carbon material each have an electrical conductivity of 2 to 5 mS/cm.~~

[Claim 913]

The method according to any one of claims 1 to 812, wherein the treatment with an activated carbon material is carried out by use of a filter containing the activated carbon material.

~~[Claim 14]~~

~~— The method according to claim 13, wherein the filter is Millistak+ Pod CR40, Zeta Plus activated carbon adsorption depth filter or SUPRAcap 50 Seitz AKS filter.~~

[Claim ~~10~~15]

The method according to any one of claims 1 to ~~9~~14, wherein the activated carbon material pool is loaded on the cation exchange material in an amount exceeding the binding capacity of the cation exchange material.

[Claim ~~11~~16]

The method according to claim ~~10~~15, wherein the activated carbon material pool is loaded on the cation exchange material at a loading density of 500 to 2000 g/L, for example.

~~{Claim 17}~~

~~— The method according to claim 16, wherein the activated carbon material pool is loaded on the cation exchange material at a loading density of 1000 to 1500 g/L.~~

[Claim ~~12~~18]

The method according to any one of claims 1 to ~~11~~17, wherein the cation exchange material is a resin, a monolith column or a membrane, for example.

~~{Claim 19}~~

~~— The method according to claim 18, wherein the cation exchange material has a functional group, which is sulfopropyl, sulfoethyl, sulfoisobutyl or carboxyl.~~

~~{Claim 20}~~

~~— The method according to claim 19, wherein the cation exchange material is POROS XS, Eshmuno CPX, Eshmuno CP-FT or CaptoS ImpAct.~~

[Claim ~~13~~21]

The method according to any one of claims 1 to ~~12~~20, wherein the solution to be loaded on the cation exchange material has a pH of 4 to 6, for example.

~~{Claim 22}~~

~~— The method according to claim 21, wherein the solution to be loaded on the cation exchange material has a pH of 4.5 to 5.5.~~

[Claim ~~14~~23]

The method according to any one of claims 1 to ~~13~~22, wherein the solution to be loaded on the cation exchange material has an electrical conductivity of 3 to 7 mS/cm, for example.

~~{Claim 24}~~

~~— The method according to claim 23, wherein the solution to be loaded on the cation exchange material has an electrical conductivity of 3 to 5 mS/cm.~~

[Claim ~~15~~25]

The method according to any one of claims 1 to ~~14~~24, wherein the cation exchange material pool is further processed through hydrophobic interaction chromatography to obtain a pool solution, for example.

~~{Claim 26}~~

~~— The method according to claim 25, wherein the hydrophobic interaction chromatography is performed in a flow-through mode, for example.~~

~~{Claim 27}~~

~~— The method according to claim 26, wherein the hydrophobic interaction chromatography is performed by use of a resin or a membrane, for example.~~

~~{Claim 28}~~

~~— The method according to claim 27, wherein the hydrophobic interaction chromatography resin or membrane has a phenyl group, a benzyl group or a hexyl group.~~

~~{Claim 29}~~

~~— The method according to claim 28, wherein the hydrophobic interaction chromatography material is POROS-Benzyl Ultra, POROS-Benzyl, Hexyl-650C, Phenyl Sepharose-6 Fast Flow, Phenyl Sepharose High Performance, Sartobind Phenyl or ReadyToProcess Adsorber Phenyl.~~

~~[Claim 1630]~~

The method according to any one of claims 1 to 914, wherein the activated carbon material pool is processed through hydrophobic interaction chromatography to obtain a pool solution, which is further processed through a cation exchange material to obtain a pool solution, for example.

~~{Claim 31}~~

~~— The method according to claim 30, wherein the hydrophobic interaction chromatography is performed in a flow-through mode, for example.~~

~~{Claim 32}~~

~~— The method according to claim 31, wherein the hydrophobic interaction chromatography is performed by use of a resin or a membrane, for example.~~

~~{Claim 33}~~

~~— The method according to claim 32, wherein the hydrophobic interaction chromatography resin or membrane has a phenyl group, a benzyl group or a hexyl group.~~

~~{Claim 34}~~

~~— The method according to claim 33, wherein the hydrophobic interaction chromatography material is POROS-Benzyl Ultra, POROS-Benzyl, Hexyl-650C, Phenyl Sepharose-6 Fast Flow, Phenyl Sepharose High Performance, Sartobind Phenyl or ReadyToProcess Adsorber Phenyl.~~

~~[Claim 1735]~~

The method according to claim 16~~any one of claims 30 to 34~~, wherein the hydrophobic interaction chromatography pool is loaded on the cation exchange material in an amount exceeding the binding capacity of the cation exchange material, for example.

~~{Claim 36}~~

~~— The method according to claim 35, wherein the hydrophobic interaction chromatography pool is loaded on~~

the cation exchange material at a loading density of 500 to 2000 g/L, for example.

~~{Claim 37}~~

~~— The method according to claim 36, wherein the hydrophobic interaction chromatography pool is loaded on the cation exchange material at a loading density of 1000 to 1500 g/L.~~

~~[Claim 1838]~~

The method according to claim 16 or 17 ~~any one of claims 30 to 37~~, wherein the cation exchange material is a resin, a monolith column or a membrane, for example.

~~{Claim 39}~~

~~— The method according to claim 38, wherein the cation exchange material has a functional group, which is sulfopropyl, sulfoethyl, sulfoisobutyl or carboxyl.~~

~~{Claim 40}~~

~~— The method according to claim 39, wherein the cation exchange material is POROS XS, Eshmuno CPX, Eshmuno CP-FT or CptoS ImpAct.~~

~~[Claim 1941]~~

The method according to any one of claims 1630 to 1840, wherein the solution to be loaded on the cation exchange material has a pH of 4 to 6, for example.

~~{Claim 42}~~

~~— The method according to claim 41, wherein the solution to be loaded on the cation exchange material has a pH of 4.5 to 5.5.~~

[Claim 2043]

The method according to any one of claims 1630 to 1942, wherein the solution to be loaded on the cation exchange material has an electrical conductivity of 3 to 7 mS/cm, for example.

~~[Claim 44]~~

~~— The method according to claim 43, wherein the solution to be loaded on the cation exchange material has an electrical conductivity of 3 to 5 mS/cm.~~

[Claim 2145]

The method according to any one of claims 1 to 2044, wherein at least one of the impurities is selected from the group consisting of host cell-derived proteins (host cell proteins; HCPs), phospholipase B-like 2 (PLBL2), viruses, virus-like particles, high-molecular weight species (HMWS), low-molecular weight species (LMWS), culture medium components, leached protein A and/or DNA.

[Claim 2246]

The method according to any one of claims 1 to 2145, wherein the steps are carried out continuously.

~~[Claim 47]~~

~~— An antibody purified by a method according to any one of claims 1 to 46.~~