

CLAIMS

1. A method of purifying recombinant acid sphingomyelinase (rASM) comprising the steps of:
 - (i) subjecting a protein mixture comprising rASM and host cell proteins (HCPs) to a cation exchange (CEX) chromatography, or
subjecting a protein mixture comprising rASM and HCPs to an immobilized metal affinity chromatography (IMAC), or
subjecting a protein mixture comprising rASM and HCPs to both a CEX chromatography and an IMAC; and
 - (ii) collecting eluate from the CEX chromatography or the IMAC, thereby obtaining a purified rASM preparation.
2. A method of modulating the relative amounts of isoforms of recombinant acid sphingomyelinase (rASM) in an initial rASM composition, wherein the initial rASM composition comprises an unmodified rASM isoform, and at least one rASM isoform having one or more modifications selected from the group consisting of C-terminus cysteinylolation, S-glutathionylation, dimerization, and truncation, wherein the method comprises
 - (i) subjecting the initial rASM composition to a cation exchange (CEX) chromatography, or
subjecting the initial rASM composition to an immobilized metal affinity chromatography (IMAC), or
subjecting the initial rASM composition to both a CEX chromatography and an IMAC; and
 - (ii) collecting eluate from the CEX chromatography or the IMAC, thereby obtaining a purified rASM preparation.
3. A method of modulating recombinant acid sphingomyelinase (rASM) specific activity in a liquid composition comprising an unmodified rASM isoform, and at least one rASM isoform having one or more modifications selected from the group consisting of C-terminus cysteinylolation, S-glutathionylation, dimerization, and truncation, wherein the method comprises:

(i) subjecting the liquid composition to a cation exchange (CEX) chromatography,
or
subjecting the liquid composition to an immobilized metal affinity
chromatography (IMAC), or
subjecting the liquid composition to both a CEX chromatography and an IMAC;
and
(ii) collecting the eluate from the CEX chromatography or the IMAC, thereby
obtaining a purified rASM preparation.

4. The method of any one of claims 1-3, wherein

the protein mixture is subjected to a CEX chromatography and an IMAC in
tandem, and eluate obtained from the CEX chromatography is subjected to the IMAC;

the protein mixture is subjected to an IMAC and a CEX chromatography in
tandem, and eluate obtained from the IMAC is subjected to the CEX chromatography; or

the protein mixture is subjected to both the CEX chromatography and the IMAC
separately, with one or more additional steps in between.

5. The method of any one of claims 1-4, where the protein mixture is subjected to one or
more additional purification columns before or after the mixture is subjected to the CEX
chromatography or the IMAC.

6. The method of any one of claims 1-5, further comprising a step of inactivating and/or
removing potential viral contaminants, and/or a step to concentrate the purified rASM.

7. The method of any one of claims 1 to 6, wherein the rASM is a recombinant human acid
sphingomyelinase (rhASM), and optionally wherein the rASM comprises the amino acid
sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

8. The method of any one of claims 1 to 7, wherein the protein mixture is obtained from
Chinese Hamster Ovary (CHO) cells expressing the rASM.

9. The method of any one of claims 1 to 8, wherein the CEX chromatography comprises a resin selected from the group consisting of carboxymethyl (CM), sulfoethyl (SE), sulfopropyl (SP), phosphate (P) and sulfonate (S).
10. The method of any one of claims 1 to 9, wherein the IMAC comprises a chelating resin, and optionally wherein the IMAC is performed with zinc, copper, or nickel.
11. The method of any one of claims 1 to 10, wherein the CEX chromatography comprises washing the CEX chromatography column with a CEX wash buffer having a first optimal pH and a first optimal salt concentration, wherein the first optimal pH and the first optimal salt concentration are predetermined depending on the resin and the starting specific activity of the protein mixture, and optionally wherein the CEX chromatography further comprises eluting the CEX chromatography column with a CEX elution buffer having a second optimal pH and a second optimal salt concentration, wherein under the second optimal pH and the second optimal salt concentration, rASM bound to the CEX chromatography column after the washing step is removed from the column.
12. The method of any one of claims 1 to 11, wherein the IMAC comprises washing the IMAC column with at least one IMAC wash buffer having a third optimal pH and a third optimal salt concentration, wherein the third optimal pH and the third optimal salt concentration are predetermined depending on the resin and the starting specific activity of the protein mixture, and optionally wherein the IMAC further comprises eluting the IMAC column with an IMAC elution buffer having a fourth optimal pH and a fourth optimal salt concentration, wherein under the fourth optimal pH and the fourth optimal salt concentration, rASM bound to the IMAC column after the washing step is removed from the column.
13. The method of any one of claims 1-12, wherein the purified rASM preparation has a specific activity of about 5 to 50 U/mg, optionally about 10 to 45 U/mg, or optionally about 10-20 U/mg.
14. The method of any one of claims 1 to 13, wherein the purified rASM preparation has an HCP level not more than 1.0 µg/mg or not more than 5.0 µg/mg.

15. The method of any one of claims 1-14, wherein the purified rASM preparation comprises rASM isoforms with modifications in total no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% of the whole rASM population, and optionally wherein the rASM isoforms with modifications comprise one or more modifications selected from the group consisting of C-terminus cysteinylation, S-glutathionylation, dimerization, and truncation.
16. The method of any one of claims 1-15, wherein the protein mixture is produced in a bioreactor having a production scale of at least 100L or at least 500L.
17. The method of any one of claims 1-16, further comprising a step to buffer exchange the purified rASM.
18. The method of any one of claims 1-17, wherein the method is conducted partially or fully under (i) refrigerated condition at 8 ± 3 °C or (ii) ambient temperature.
19. A recombinant acid sphingomyelinase (rASM) preparation comprising an unmodified rASM isoform and at least one rASM isoform species having one or more modifications selected from the group consisting of C-terminus cysteinylation, S-glutathionylation, dimerization, and truncation, wherein the unmodified rASM isoform is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% or more of the total rASM population in the rASM preparation.
20. The rASM preparation of claim 19, wherein all modified rASM isoforms in total are no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 14%, no more than 13%, no more than 12%, no more than 11%, no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5% or less of the total rASM population in the rASM preparation.
21. The rASM preparation of claim 19 or 20, wherein the rASM isoform having C-terminus cysteinylation is no more than 10%, no more than 9%, no more than 8%, no more than 7%, no more than 6%, no more than 5% or less of the total rASM population in the rASM preparation.

22. The rASM preparation of any one of claims 19-21, wherein the rASM isoform having C-terminus S-glutathionylation is no more than 5%, no more than 4%, no more than 3%, no more than 2%, no more than 1% or less of the total rASM population in the rASM preparation.
23. The rASM preparation of any one of claims 19-22, wherein the rASM isoform having C-terminus dimerization is no more than 0.2% or no more than 0.1% of the total rASM population in the rASM preparation.
24. The rASM preparation of any one of claims 19-23, wherein the rASM isoform having C-terminus truncation is no more than 8%, no more than 7%, no more than 6%, no more than 5%, no more than 4%, no more than 3% or less of the total rASM population in the rASM preparation.
25. The rASM preparation of any one of claims 19-24, wherein the rASM preparation has a purity of at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or more.
26. The rASM preparation of any one of claims 19-25, wherein the rASM preparation has a specific activity of about 5 to 50 U/mg, optionally about 10 to 20 U/mg, and/or a host cell protein (HCP) level not more than 5.0 µg/mg.
27. An rASM preparation manufactured using the method of any one of claims 1-18.
28. A pharmaceutical composition prepared by using the rASM preparation of any one of claims 19-27.
29. Use of the pharmaceutical composition of claim 28 in the manufacture of a medicament for treating acid sphingomyelinase deficiency in a subject in need thereof.