Claims

1. A method for producing purified recombinant human acid alpha-glucosidase (rhGAA), wherein the purified rhGAA comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 2, the method comprising:

in a bioreactor, culturing host cells that secrete the rhGAA;

removing media from the bioreactor;

filtering the media to provide a filtrate;

loading the filtrate onto an anion exchange chromatography (AEX) column to capture the rhGAA;

eluting the rhGAA from the AEX column;

loading the rhGAA eluted from the AEX column onto an immobilized metal affinity chromatography (IMAC) column; and

eluting the rhGAA from the IMAC column.

2. The method of claim 1, wherein the purified rhGAA comprises a first potential N-glycosylation site, a second potential N-glycosylation site, a third potential N-glycosylation site, a fourth potential N-glycosylation site, a fifth potential N-glycosylation site, a sixth potential N-glycosylation site, and a seventh potential N-glycosylation site.

3. The method of claim 1, further comprising:

loading the rhGAA eluted from the IMAC column onto a third chromatography column; and

eluting the rhGAA from the third chromatography column.

4. The method of claim 3, wherein the third chromatography column is selected from a cation exchange chromatography (CEX) column and a size exclusion chromatography (SEC) column.

5. The method of claim 3, wherein filtering the media is selected from alternating tangential flow filtration (ATF) and tangential flow filtration (TFF).

6. The method of claim 3, further comprising inactivating viruses in one or more of the rhGAA eluted from the AEX column, the rhGAA eluted from the IMAC column, and the rhGAA eluted from the third chromatography column.

7. The method of claim 3, further comprising filtering the rhGAA eluted from the IMAC column or the rhGAA eluted from the third chromatography column to provide a filtered product and filling a vial with the filtered product.

8. The method of claim 7, further comprising lyophilizing the filtered product.

9. The method of claim 1, wherein the host cells comprise Chinese hamster ovary (CHO) cells.

10. The method of claim 1, wherein:

(i) at least 90% of the purified rhGAA binds to cation-independent mannose-6-phosphate receptor (CIMPR); or

(ii) at least 90% of the purified rhGAA comprises an N-glycan carrying mono-mannose-6-phosphate (mono-M6P) or bis-mannose-6-phosphate (bis-M6P).