# Request to amend the list of processing aids for the production of Steviol Glycosides under Australia and New Zealand Food Standard Code Standard 1.3.1– Food Additives and Standard 1.3.4 – Identity and Purity

1 January 2023

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## A. General Requirements

The following general information as required by Section 3.1.1 - General Requirements of the Food Standards Australia New Zealand (FSANZ) Application Handbook (FSANZ, 2019) is provided and addressed in Section A below.

### A.1 Format of the Application

The format of this application follows the numbering and the headings found in the Application Handbook updated 28 May 2020. Appendix A contains the details of the genetic modifications to produce the enzymes and Appendix B contains information on Reb M and I produced by this enzyme system.

### A.2 Applicant Details

Manus Bio leverages rapid advances in biology to produce complex natural ingredients used in our daily lives as flavors, fragrances, food ingredients, cosmetics, vitamins, pharmaceuticals, and agricultural chemicals. Using its advanced fermentation technology, Manus Bio recreates natural processes for next-generation industrial biomanufacturing and provides sustainable, cost-effective sources of ingredients for health, wellness, and nutrition. We call it Biomanufacturing Redefined™.

**Corporate Address:**

**Manufacturing Location:**

**Correspondence should be addressed to**

| Name | Email | Contact |
|---|---|---|

## A.3 Purpose of the Application

Requested Amendments to FSANZ Food Code Schedule 18: 1) The list of processing aids in Schedule 18 used to produce steviol glycosides by biotransformation include the enzyme UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) produced by *Escherichia coli* expressing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum*, in addition to the glucosyltransferases (EC 2.4.1.17) and sucrose synthase (EC 2.4.1.13) already listed, 2) For glucosyltransferases, include *E. coli* modified with genes from *Oryza sativa* in addition to the other sources already listed (*Stevia rebaudiana* and *Solanum tuberosum),* 3)For sucrose synthases, include *E. coli* modified with genes from *Glycine max* in addition to the other sources already listed (*Arapidopsis thaliana*).

**Amending Schedule S3-35**

**Specification for steviol glycosides produced by enzymatic conversion**

(1) In this section:

***prescribed Rebaudiosides*** are:

(a) Rebaudioside D

(b) Rebaudioside M; and

(c) Rebaudioside AM.

(d) Rebaudioside I

……..

(c) by enzymatic conversion of purified stevia leaf extract to produce one or more prescribed Rebaudiosides using a combination of enzymes that contains:

(i) a UDP-glucosyltransferase from *Stevia rebaudiana* sourced from *Escherichia coli*; and /or

(ii) a UDP-glucosyltransferase from *Solanum lycopersicum* sourced from *Escherichia coli*; and/or

(iii) a UDP-glucosyltransferase from *Oryza sativa* sourced from *Escherichia coli*; and/or

(iv) a sucrose synthase (EC 2.4.1.13) sourced from *Escherichia coli* and/or

(v) a UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) sourced from *Escherichia coli*

The Standard 1.3.1 does not need to be amended since it includes all steviol glycosides found in leaf extract nor does Specification S3-36, Steviol Glycosides.

## A.4 Justification for the Application

### A.4.1 Enable Innovation and Competition in the Marketplace

The change would permit innovation and competition in the marketplace. Increased competition in the marketplace for sweeteners could lead to wider availability to both industry and consumers, potentially reducing the cost of non-caloric sweeteners. Manus

Bio's cost of manufacturing is more efficient and results in less environmental burden and lower costs to the environment than sugar due to its efficiency and potency.

### A.4.2 Facilitate Healthier Products for Consumers

The change will also allow for increased use of steviol glycosides in better tasting products that have fewer calories to combat the obesity and diabetes epidemic. High potency sweeteners contribute less metabolic energy on a sweetness basis. The presence of a widely available natural sweetener tasting like sugar, will enable more consumer choice of products at lower sugar levels than are available today. In addition, low-calorie sweeteners that do not increase blood sugar levels provide options for diabetics trying to reduce or limit their intake of sugar and carbohydrates.

### A.4.3 Cost and Benefits of the Application

The advantages and benefits are listed above in A.4.1 and A.4.2. There are no known disadvantages to permit this change. The change does not have any safety or environmental disadvantages.

Manus Bio submitted a U.S. Food and Drug Administration GRAS modification (GRAS #1010) (Manus Bio, 2020) and received a letter of no questions dated 26 January 2022 (U.S. FDA, 2022).

## A.5 Information to Support the Application

Information to support the application is presented in detail in Section C.6 Potential Allergenicity of the Enzymes.

## A.6 Assessment Procedure

Manus Bio considers this a general procedure assessment. This change involves minor amendments to the lists of processing aids 1.3.1 Food Additives and Standards 1.3.4 Identity and Purity.

### A.7 Confidential Commercial Information (CCI)

All confidential commercial information is contained in Appendix A. Manus Bio considers this information to be proprietary and of a sensitive business nature and formally requests it be kept confidential.

The confidential commercial information found in Appendix A can be summarized as the details of the genetic modification made to *E. coli K12* to make the enzymes necessary to biotranform steviol glycosides into Reb M and Reb I. It is confidential commercial information. A summary of this information is in Section B. Processing Aids and is not confidential.

### A.8 Other confidential information

No additional information is considered confidential by Manus Bio.

### A.9 Exclusive Capturable Commercial Benefit

Manus Bio expects that this application will confer capturable benefit due to its proprietary nature.

### A.10 International Standards and National Standards

#### A.10.1 The Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Manus Bio's Reb M and Reb I meet the JECFA, 84$^{th}$ meeting 2017 FAO JECFA Monographs 20 with the exception of the method of manufacture listed in Annex A.

#### A.10.2 The United States

Manus Bio's steviol glycosides are GRAS in the United States. Manus Bio submitted a GRAS notification under GRAS 1010 (Manus Bio, 2020) and received a letter of no questions dated 26 January 2022 (U.S. FDA, 2022).

## A.11 Statutory Declaration

Signed Statutory Declarations for Australia and New Zealand are provided in Appendix C.

## A.12 Checklists

Completed checklists relating to the information required for submission with this application based on the relevant guidelines in the FSANZ Application Handbook are provided in Appendix D and E.

## B. Technical Information

### B.1. Processing Aids

Confidential information is in Appendix A and summarized as follows:

The list of processing aids in Schedule 18 used to produce steviol glycosides by biotransformation include the enzyme UTP-glucose-1-phosphate uridylyltransferase (EC 2.7.7.9) produced by *Escherichia coli* expressing the gene for UTP-glucose-1-phosphate uridylyltransferase from *Bifidobacterium bifidum*, in addition to the glucosyltransferases (EC 2.4.1.17) and sucrose synthase (EC 2.4.1.1) already listed. For glucosyltransferases, include *E. coli* modified with genes from *Oryza sativa* in addition to the other sources already listed (*Stevia rebaudiana and Solanum tuberosum)*. For sucrose synthases, include *E. coli* modified with genes from *Glycine max* in addition to the other sources already listed (*Arapidopsis thaliana*).

### B.2 Parental Strain

The parental strain *E. coli* K-12 sub-strain MG1655 Fnr- was obtained from the *E. coli* Genetic Stock Center (CGSC) and is currently listed under the designation CGSC 6300.

### B.3 Production Strain

The parental strain *E. coli* K-12 was engineered to express enzymes (UDP-glucosyl transferases) for the glycosylation of steviol glycosides and to improve the overall production efficiency of Rebaudioside M. In addition, the strain was engineered to increase the supply of uridine diphosphate glucose (UDP-Glu), a precursor required for glycosylation of steviol glycosides through a series of gene deletions and overexpressions. All heterologously overexpressed genes originated from biosafety level 1 organisms that are not associated with any known allergens or toxins, including *Stevia rebaudiana*, *Oryza sativa*, *Glycine max*, and *Bifidobacterium bifidum*.

### B.4 Engineering Methods

Overexpressed genes were synthesized, codon-optimized for *E. coli*, and introduced into stable, non-essential regions of the genome via standard techniques utilizing homologous recombination with positive selection and counterselectable markers (Datsenko and Wanner, 2000). These regions include but are not limited to endA, recA, and araA. All selection markers

used during the engineering process were removed, and no antibiotic resistance markers are present in the final production strain.

Gene deletions were generated using standard techniques utilizing homologous recombination with positive selection and counterselectable markers (Datsenko and Wanner, 2000). All selection markers used during the engineering process were removed, and no antibiotic resistance markers are present in the final production strain.

The identity of the final production strain was confirmed by Sanger sequencing of modified regions and by whole genome sequencing.

## C. Information Related to the Safety of the Food Additive

### C.1 History of safe use of *E. coli* K-12

*E. coli* K-12 is a non-pathogenic and non-toxigenic organism belonging to Biosafety Level 1 according to the National Institutes of Health (NIH, 2016). Additional supporting evidence for the safety of *E. coli* K-12 is cited in other GRAS notices to FDA. *E. coli* K-12 has a long history of safe use in the industrial production of specialty chemicals and human drugs (U.S. EPA, 1997). For example, a food enzyme preparation (chymosin) obtained from a genetically modified *E. coli* K-12 strain was affirmed as GRAS by the FDA in 1990 (Flamm, 1991; Olempska-Beer et al., 2006) and has been used safely for cheese production worldwide. *E. coli* also serves as a host for the production of enzymes currently used in an approved process for the enzymatic conversion of steviol glycosides (GRN745) (U.S. FDA, 2018).

### C.2 Residual Protein Analysis

#### C.2.1 Reb M

To provide an indication of the removal of residual protein in the final product, 3 non-consecutive batches of the high-purity Rebaudioside M (≥95% Rebaudioside M) produced by enzymatic conversion of steviol glycosides (Lot Numbers MAM060420, MAM060520E, MAM061120A) were analyzed using the bicinchoninic acid (BCA) assay. Samples were reconstituted to a concentration of 1 g/L and measured using a 96 well plate format BCA assay on a Tecan Infinite M1000 Pro plate reader. The limit of detection was 22.5 ppm on a w/v basis.

The results of the analysis were below the limit of detection, which provides further evidence that downstream processing successfully removed residual proteins from the final product.

### C.2.2 Reb I

To confirm the removal of residual protein from the final product, 3 non-consecutive batches of the high- purity Rebaudioside I (≥95% Rebaudioside I) (Lot Numbers A003, 002A, and 002C) were analyzed using the bicinchoninic acid (BCA) assay method. Samples were reconstituted to a concentration of 1 g/L and measured using a 96 well plate format BCA assay on a Tecan Infinite M1000 Pro plate reader. The limit of detection was 22.5 ppm on a w/v basis. The results of the analysis were below the limit of detection, which provides further evidence that downstream processing successfully removed residual proteins from the final product.

## C.3 Residual DNA Analysis

### C.3.1 Reb M

To confirm the absence of residual DNA in Reb M, 3 non-consecutive lots of final products (Lot Numbers MAM060520E, MAM200716J, MAM060420A) were assayed by polymerase chain reaction (PCR). Primer design and PCR conditions were designed to amplify a specific fragment of one of the genes inserted in the production strain. Extracted genomic DNA containing the selected gene was used as a positive control. Reb M samples were prepared in aqueous solution and assayed by PCR at a final concentration of 100 mg/L. Samples were assayed with or without positive control DNA at a final concentration of 1 ng/µL. A standard curve was prepared from 1 ng/µL to 0.1fg/µL to determine the limit of detection of the assay (320 fg/µL). All samples with a positive control spike showed an expected PCR product, whereas all the Reb M samples without a genomic DNA spike showed no product. The gel images were analyzed using ImageJ for quantitative analysis. Background subtracted mean grey values from each of the samples were fit to values from the standard curve and found to be below the limit of detection of the assay (Table 2.3.4a). These results confirm the absence of residual genomic DNA in the Reb M final product above the analytical limit of detection of 320 fg/µL.

**Table C.3.1 Absence of residual DNA: Reb M**

| Sample | Background Subtracted Mean Grey Value | Calculated Concentration (ng/µl) |
|---|---|---|
| MAM060420A | -0.877 | below LOD* |
| MAM060520E | -0.771 | below LOD* |
| MAM200716J | -0.431 | below LOD* |

*LOD = limit of detection

### C.3.2 Reb I

To confirm the absence of residual DNA in Rebaudioside I, 3 non-consecutive lots of final product (Lot Numbers A003, 002A, and 002C) were assayed by polymerase chain reaction (PCR). Primer design and PCR conditions were designed to amplify a specific fragment of one of the genes inserted in the production strain. Extracted genomic DNA containing the selected gene was used as a positive control. Reb I samples were prepared in aqueous solution and assayed by PCR at a final concentration of 100 mg/L. Samples were assayed with or without positive control DNA at a final concentration of 1 ng/µL. A standard curve was prepared from 1 ng/µL to 0.1fg/µL to determine the limit of detection of the assay (320 fg/µL). All samples with a positive control spike showed an expected PCR product, whereas all the Reb I samples without a genomic DNA spike showed no product. The gel images were analyzed using ImageJ for quantitative analysis. Background subtracted mean grey values from each of the samples were fit to values from the standard curve and found to be below the limit of detection of the assay (Table 2.3.4b). These results confirm the absence of residual genomic DNA in the Reb I final product above the analytical limit of detection of 320 fg/µL.

**Table C.3.2 Absence of residual DNA: Reb I**

| Sample | Background Subtracted Mean Grey Value | Calculated Concentration (ng/µL) |
|---|---|---|
| A003 | -2.219 | below LOD* |
| 002A | 1.043 | below LOD* |
| 002C | 1.246 | below LOD* |

*LOD = limit of detection

### C.4 Pesticide Residue

A sample of the starting steviol glycoside material was extracted by a QuEChERs method and a combined LC & MS / GC & MS screening for pesticide residue was performed. The starting stevia leaf material (≥90% total steviol glycosides; Lot # 202003051 and Lot #201901171) demonstrated the absence of residues of commonly used pesticides in the final product.

### C.5 Pathogenicity/Toxicogenicity of the Parental Strain

As discussed in GRN 745, *E. coli* K-12 is not considered a human or animal pathogen and has been classified as Biosafety Level 1 according to the NIH Guidelines (NIH, 2016). *E. coli* K-12 is often used as a reference organism when investigating the virulence factors of pathogenic *E. coli* strains as it is non-pathogenic (BlancPotard et al., 2002; Kaper et al., 2004). This species and its derivatives are unable to colonize the mammalian gastrointestinal tract, and do not produce toxins such as Shiga toxin, and are unable to persist in the soil and water (Bogosian et al., 1996; U.S. EPA, 1997). As previously described, the parental strain does not carry any introduced antibiotic resistance genes and the complete genome of this strain has been sequenced, confirming the absence of any toxigenic potential (Blattner et al., 1997; Hayashi et al., 2006).

### C.6 Potential Allergenicity of the Enzymes

The potential allergenicity of all non-native enzymes was investigated using an in-silico approach. A sequence homology search was conducted according to the approach outlined by the Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) (2001) and the WHO/FAO (2009) using the Allergen Online Database Version 20 (available at http://www.allergenonline.org; updated February 2020) maintained by the Food Allergy Research and Resource Program (FARRP) of the University of Nebraska (FARRP, 2020). This was done to confirm that the enzymes do not contain amino acid sequences similar to other known allergens that might produce an allergenic response. The database contains a comprehensive list of putative allergenic proteins developed via a peer reviewed process for the purpose of evaluating food safety. No matches were identified from searching with the full amino acid sequence for each enzyme. According to the FARRP guidelines, an identity threshold of greater than 50% or an E-score lower than $1x10^{-7}$ suggest cross reactivity with the known allergen to be a possibility. A second homology search was conducted according to the approach outlined by the FAO/WHO (2001) and the WHO/FAO (2009). In accordance with this guideline, the Allergen Online database was searched using a sliding window of 80-amino acid

sequences (segments 1-80, 2-81, 3-82, etc.) derived from the full-length amino acid sequence for each enzyme. The 80-amino acid alignment search was conducted using default settings (E value cutoff = 1 and maximum alignments of 20). Significant homology is defined as an identity match of greater than 35% (Codex Alimentarius, 2009). Using this search strategy, again no matches were identified and the level of protein in the final product NutraSweet M™ is reported as below the limit of detection

Manus Bio submitted a GRAS notification (#1010) and received a letter of no objection dated 26 January 2022 (U.S. FDA, 2022).

## D. Information Related to the Dietary Exposure to the Food Additive

There is no dietary exposure to the enzyme in Rebaudioside M or I. The Reb M and Reb I are not a genetically modified food and not derived from an organism that has been modified using gene technology.

The resulting product is both PCR negative and negative for residual protein.
Steviol glycosides have been approved for years and this amendment should not significantly affect consumption. No additional food groups are requested for steviol glycosides in this application.

## Appendix A  Confidential Information

All confidential commercial information is contained in Appendix A attached separately. Manus Bio considers this information to be proprietary and of a sensitive business nature and formally requests it be kept confidential.

# Appendix B Nature and Technological Purpose Information

## B.1 Method of Manufacturing for Reb M and Reb I

### B.1.1 Raw Materials and Processing Aids

All raw materials, processing aids (Table B.1.1), and purification equipment used to manufacture Manus Bio's Rebaudioside M through bioconversion of steviol glycosides are food-grade ingredients, permitted by U.S. regulation, have GRAS status, or have been self-affirmed as safe for use in food for their respective uses. The food-grade ingredients are compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (e.g., JECFA, CODEX, United States Pharmacopeia, and European Pharmacopoeia).

The starting material for fermentation is a water/ethanol extract of *S. rebaudiana* sold as a food ingredient by a third-party supplier. This extract is produced in a facility with SQF, GMP, ISO9001, ISO 14001, HACCP, HALAL and Kosher certifications. In brief, steviol glycosides are extracted from the stevia leaf by a series of crushing, dissolution, solvent extraction, and precipitation steps that are consistent with the methodology outlined in the CTA for steviol glycosides (FAO, 2016). The steviol glycoside extract contains ≥90% total steviol glycosides. Because the product is specified to contain only ≥ 90% total steviol glycosides, it does not meet the purity requirements specified by JECFA (Joint European Committee on Food Additives, 2016; Joint FAO/WHO Expert Committee on Food Additives, 2016); however, the steps to getting to the highly purified steviol glycosides (≥ 95%) is permitted. In this process the glycosides are purified to >95% purity which meets the joint FAS/WHO requirements.[1]

[1] Compliant with the specifications set forth in the Food Chemicals Codex or equivalent international food or pharmacopeia standard (e.g., JECFA, CODEX, United States Pharmacopeia, and European Pharmacopoeia).

## Table B.1.1 Materials used in production process

| Material | | Use | 21CFR (U.S. FDA 2018) | Approved Use |
|---|---|---|---|---|
| Glucose Monohydrate | C6H14O7 | Medium nutrient | GRAS | GRAS when used in accordance with cGMP |
| Ammonium sulfate | (NH4)2SO4 | Medium nutrient | 582.1143/184.1443 | GRAS when used in accordance with cGMP |
| Potassium phosphate monobasic | KH2PO4 | Medium nutrient | 160.110 | GRAS when used in accordance with cGMP |
| Potassium phosphate dibase | K2HPO4 | Medium nutrient | 160.110 | GRAS when used in accordance with cGMP |
| Citric Acid | C6H8O7 | Medium nutrient | 184.1033 | pH control agent & processing aid with no limitation other than cGMP |
| Yeast Extract | | Medium nutrient | 184.1983 | GRAS when used in accordance with cGMP |
| Antifoam | | media | 173.340 | Processing when used in according with cGMP. Secondary direct food additive, defoaming agent |
| Manganese (II) chloride tetrahydrate | MnCl2.4H2O | Medium nutrient | 582.5446/184.1446 | GRAS when used in accordance with cGMP |
| Zinc sulfate monohydrate | ZnSO4.H2O | Medium nutrient | 582.5997/182.8997 | GRAS when used in accordance with cGMP |
| Copper (II) chloride pentahydrate | Cl2CuH10O5 | Medium nutrient | 184.1261 | GRAS when used in accordance with cGMP |
| Calcium chloride dihydrate | CaCl2.2H2O | Medium nutrient | 582.1193/582.6193/184.1193 | GRAS when used in accordance with cGMP |
| Peptone | | Medium nutrient | | |
| Sodium molybdate dihydate | Na2MoO4.2H2O | Medium nutrient | 40CFR 180.920 | GRAS when used in accordance with cGMP |
| Iron (II) sulfate heptahydrate | FeSO4.7H2O | Medium nutrient | 184.1315 | GRAS when used in accordance with cGMP |
| Magnesium sulfate Heptahydrate | H14MgO11S | Medium nutrient | 582.5443/184.1443 | Nutrient supplement with no limitation when used in accordance with cGMP |
| Thiamine Hydrochloride | C12H18Cl2N4OS | Medium nutrient | 582.5875/184.1875 | GRAS when used in accordance with cGMP |
| Stevia extract | | media | 170.30 | GRAS when used in accordance with cGMP |
| 190 Proof Ethanol | CH3CH2OH | Elution Solvent Crystallization | 184.1293 | GRAS when used in accordance with cGMP |
| Sucrose | C12H22O11 | Medium nutrient | GRAS | GRAS when used in accordance with cGMP |
| Sodium Hydroxide | NaOH | Medium nutrient | 184.1205 | GRAS when used in accordance with cGMP |
| Sulfuric Acid | H2SO4 | Medium Nutrient | 184.1293 | GRAS when used in accordance with cGMP |
| Sodium Benzoate | C7H5NaO2 | Preservative | 184.1733 | Used as an antimicrobial agent at levels not to exceed GMP (typically 0.1% in food) |
| Potassium Sorbate | C6H7KO2 | Preservative | 182.364 | GRAS when used in accordance with cGMP |
| Sodium Carbonate Monohydrate | CH2NA2O4 | Medium Nutrient | 184.1742 | GRAS when used in accordance with cGMP |
| Glycerin | C3H8O3 | Medium Nutrient | 182.132 | GRAS when used in accordance with cGMP |
| Potassium Chloride | KCL | Medium Nutrient | 184.1622 | GRAS when used in accordance with cGMP |
| Adsorption resin | | Purification | 173.65 | GRAS when used in accordance with cGMP |
| Ion-exchange resin | | Purification | 173.25 | GRAS when used in accordance with cGMP |
| Activated carbon | | Decolorizing agent | GRAS | Used in accordance with cGMP |
| Microfiltration / Purification | | Ultrafiltration | 177.291 | Used in accordance with cGMP |
| Boiler Chemicals | | Anticorrosion | 173.31 | Used in accordance with cGMP (non contact) |

### B.1.2 Manufacturing Process

#### B.1.2a Reb M

Manus Bio's high-purity Rebaudioside M (≥95% Rebaudioside M) is produced by bioconversion of steviol glycosides using an *E. coli* strain derived from *E. coli* K-12. The production strains are grown in media containing steviol glycoside extracts prepared from the leaves of *S. rebaudiana Bertoni* in accordance with the methodology outlined in the Chemical and Technical Assessment (CTA) for steviol glycosides (FAO, 2016). Cells mediate the glycosylation of steviol glycosides in the leaf extract to Rebaudioside M. The broth is then sterilized and centrifuged to separate biomass from the aqueous phase. Rebaudioside M is extracted from the aqueous phase and purified through crystallization and washes. Reb M crystals are then dried and milled to a final product containing ≥95% Reb M. The purification processes described are consistent with the methodologies for the manufacture of steviol glycosides as described in the CTA published by FAO/JECFA (FAO, 2016). Manus Bio's ≥95% Rebaudioside M, produced by bioconversion of steviol glycosides, is manufactured in a facility registered as a FDA Food Facility. The plant operates under cGMPs (Good Manufacturing Practices) outlined in the Food Safety Modernization Act 21 CFR 117, including required HACCP and Food Defense Plans, and is subject to audit from regulatory authorities including the US FDA and GA department of Agriculture. Manufacturing shall be certified to a GFSI (Global Food Safety Initiative) compliant audit scheme.

#### B.1.2b Reb I

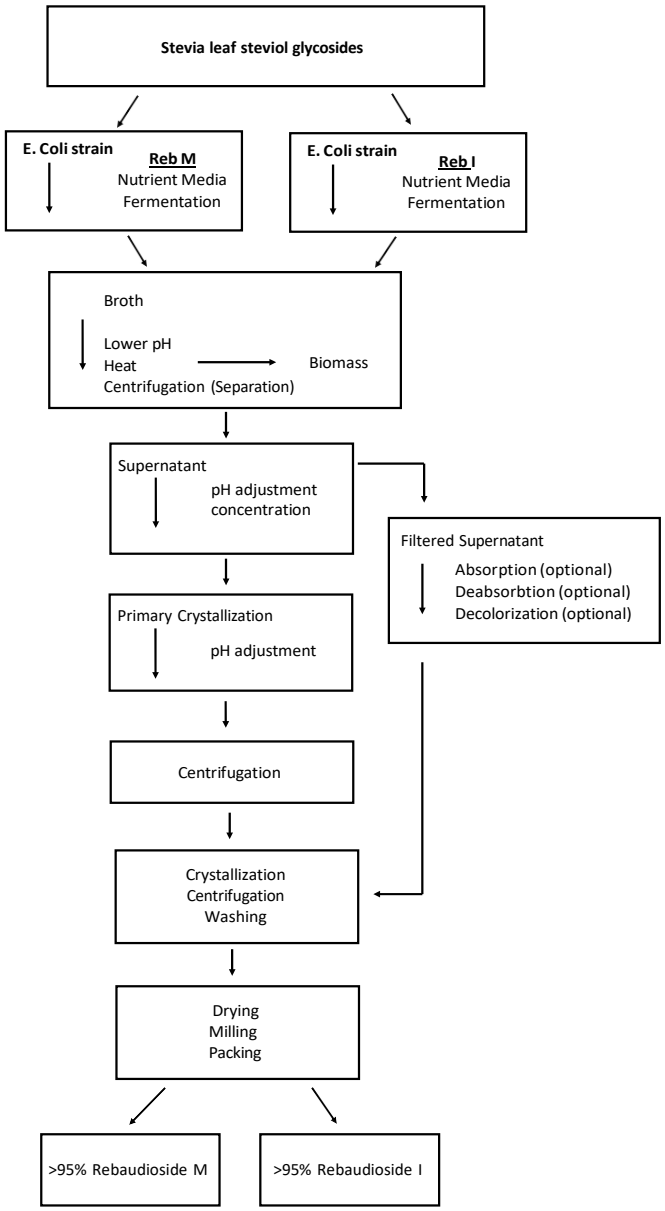
Manus Bio's high-purity Rebaudioside I (≥95% Rebaudioside I) is produced by enzymatic conversion of steviol glycosides using an *E. coli* strain derived from *E. coli* K-12. The production strains are grown in media containing steviol glycoside extracts prepared from the leaves of *S. rebaudiana* Bertoni in accordance with the methodology outlined in the Chemical and Technical Assessment (CTA) for steviol glycosides (FAO, 2016). Specifications for the starting material are provided in Table B.1.2b. Within the growth medium containing stevia leaf extract, UGT enzymes produced by the *E. coli* K-12 cells mediate the glycosylation of steviol glycosides to Rebaudioside I. After sufficient Rebaudioside I has been produced, the media and *E. coli* K-12 biomass is heat inactivated and the broth clarified to remove the inactivated biomass and enzymes. Rebaudioside I is then purified using physical processing steps including filtration, aqueous crystallization, centrifugation, rinsing and drying using typical food processing equipment and steps. Dried Rebaudioside I may be in a crystalline or amorphous solid form and may be further milled, spray dried, freeze dried, agglomerated, compacted, and granulated or

other physical form modification to achieve a desirable particle size of the final product, ≥95% Rebaudioside I. The purification processes described are consistent with the methodologies for the manufacture of steviol glycosides as described in the CTA published by FAO/JECFA (FAO, 2016). Figure B.1.2 illustrates the manufacturing process.

**Table B.1.2b Specification for Steviol Leaf Extract Starting Material**

| Items | Standard | Test Method |
|---|---|---|
| **Appearance** | White powder | Visual Check |
| **Steviol glycosides** | NLT 90.0% | HPLC |
| **Specific Optical Rotation** | ~-30° ~ -38° | Polarimeter |
| **Specific Absorbance** | NMT 0.2 | Polarimeter |
| **Loss on Drying** | NMT 3.0% | JECFA VOL. 4 |
| **Residue on Ignition** | NMT 0.20% | AOAC 945.46 |
| **Heavy Metal** | NMT 10 ppm | ICP MS AOAC |
| **Arsenic** | NMT 1 ppm | ICP MS AOAC |
| **Mercury** | NMT 0.1 ppm | ICP MS AOAC |
| **Lead** | NMT 3.0 ppm | ICP MS AOAC |
| **Cadmium** | NMT 1.0 ppm | ICP MS AOAC |
| **Total Plate Count** | NMT 1000 cfu/g | AOAC 966.23 |
| **Yeast and Mold** | NMT 100 cfu/g | AS 1766.2.2 |
| ***E. Coli*** | Negative | ISO7251 |
| **Pathogenic Bacteria** | Negative | ISO7251 |
| **Salmonella** | Negative | ISO6579 |
| **Staphylococcus** | Negative | FDA/BAM Online Chap. 12 |
| **Residual of Solvent Ethanol** | 300 ppm max | USP31 <467> |
| **Residual of Solvent Methanol** | 100 ppm max | USP31 <467> |

## Figure B.1.2 Manufacturing process of Manus Bio's Rebaudioside M and Rebaudioside I produced by bioconversion



## B.2 Product Specifications and Batch Analyses

### B.2.1 Reb M

Appropriate food-grade specifications have been established for high-purity Rebaudioside M (≥95% Rebaudioside M) based on the specifications for steviol glycosides established by JECFA (2017a) (Table B.2.1). All analytical methods used to measure each specification parameter are internationally recognized methods (e.g., United States Pharmacopeia [USP], Association of Official Analytical Chemists [AOAC], or JECFA). Total steviol glycoside content is measured using the high-performance liquid chromatography (HPLC) method described in the JECFA specification monograph for steviol glycosides from S. rebaudiana Bertoni (JECFA, 2017a, b).

**Table B.2.1 Product Specifications for High-Purity Rebaudioside M (≥95% Rebaudioside M)**

| ***Physical and Chemical Parameters*** | | | |
|---|---|---|---|
| **Specification Parameter** | **High-Purity Rebaudioside M (≥95% Rebaudioside M)** | **Current JECFA Specifications for Steviol Glycosides (JECFA, 2017a)** | **Method of Analysis** |
| Total steviol glycosides (anhydrous basis) | ≥95% | ≥95% total steviol glycosides[a] | TN34236 [Monograph 19 (82nd JECFA Meeting 2016)] |
| Rebaudioside M | ≥95% | N/A | TN34240 [Monograph 19 (82nd JECFA Meeting 2016)] |
| Loss on drying | ≤6% | ≤6% (105°C, 2h) | TN46040 (CRA E-46) |
| pH (1% solution) | 4.5 to 7.0 | 4.5 to 7.0 | TN60730 (AOAC 981.12) |
| Residual ethanol | ≤5,000 ppm (≤0.5%) | ≤0.5% | TN64080 (USP 32-NF 27) |
| Residual methanol | ≤200 ppm (≤0.02%) | ≤0.02% | TN64080 (USP 32-NF 27) |
| Total ash | ≤1% | ≤1% | TN09560 (AOAC 900.02) |
| Lead | ≤1 ppm | ≤1 ppm | TN44290 (AOAC 993.14) |
| Arsenic | ≤1 ppm | ≤1 ppm | TN44292 (AOAC 993.14) |
| Cadmium | ≤1 ppm | NS | TN44291 (AOAC 993.14) |
| Mercury | ≤1 ppm | NS | TN44293 (AOAC 993.14) |

**Table B.2.1 Product Specifications for High-Purity Rebaudioside M (≥95% Rebaudioside M)**

***Microbiological Parameters***

| Specification Parameter | High-Purity Rebaudioside M (≥95% Rebaudioside M) | Current JECFA Specifications for Steviol Glycosides (JECFA, 2017a) | Method of Analysis |
|---|---|---|---|
| Total plate count | <1,000 CFU/g | <1,000 CFU/g | TN10560 (CRA Microbiological Methods I-A) |
| Mold | <100 CFU/g | <200 CFU/g | TN47010 (CRA Microbiological Methods II-A-1) |
| Yeast | <100 CFU/g | <200 CFU/g | TN97010 (CRA Microbiological Methods I-A) |
| Coliforms | <3 MPN/g | NS | TN10510 (CRA Microbiological Methods IV-B) |
| *Escherichia coli* | Not detected | Not detected | TN10512 (CRA Microbiological Methods IV-B) |
| *Salmonella* | Negative/25 g | Not detected | TN10547 (CRA Microbiological Methods V-A) |

AOAC = Association of Official Analytical Chemists; CFU = colony-forming units; CRA = Corn Refiners Association; JECFA = Joint FAO/WHO Expert Committee on Food Additives; MPN = most probable number; N/A = not applicable; NS = not specified; ppm = parts per million; USP = United States Pharmacopeia.

^Where steviol glycosides "consists of a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni including, glucose, rhamnose, xylose, fructose, deoxyglucose, galactose, and arabinose". (JECFA, 2017a).

### B.2.2 Reb I

Appropriate food-grade specifications have been established for high-purity Rebaudioside I (≥95% Rebaudioside I) which meet or exceed the specifications for steviol glycosides established by JECFA (2017a) (Table B.2.2). All analytical methods used to measure each specification parameter are internationally recognized methods (e.g., United States Pharmacopeia [USP], Association of Official Analytical Chemists [AOAC], or JECFA). Total steviol glycoside content is measured using the high-performance liquid chromatography (HPLC) method described in the JECFA specification monograph for steviol glycosides from S. rebaudiana Bertoni (JECFA, 2017a, b)

**Table B.2.2 Product Specifications for High-Purity Rebaudioside I (≥95% Rebaudioside I)**

| Specification Parameter | Specification |
|---|---|
| Appearance | White-to-off-white powder |
| Total steviol glycosides (anhydrous basis) | ≥95% |
| Rebaudioside I | ≥95% |
| Loss on drying | ≤6% |
| pH (1% solution) | 4.5 to 7.0 |
| Residual ethanol | ≤5,000 ppm |
| Residual methanol | ≤200 ppm |
| Total ash | ≤1% |
| Lead | ≤1 ppm |
| Arsenic | ≤1 ppm |
| Cadmium | ≤1 ppm |
| Mercury | ≤1 ppm |
| Total plate count | <1,000 CFU/ g |
| Mold | <100 CFU/ g |
| Yeast | <100 CFU/ g |
| Coliforms | <3 M PN/ g |
| Escherichia coli | Not detected |
| Salmonella | Negative/ 25 g |

CFU = colony-forming units; MPN = most probable number; ppm = parts per million

^ Where steviol glycosides "consists of a mixture of compounds containing a steviol backbone conjugated to any number or combination of the principal sugar moieties in any of the orientations occurring in the leaves of *Stevia rebaudiana* Bertoni including glucose, rhamnose, xylose, fructose, deoxyglucose, galactose, and arabinose" (JECFA, 2017a).

## B.3 Batch Analyses

### B.3.1 Reb M

Analysis of 3 non-consecutive lots of high-purity Rebaudioside M (≥95% Rebaudioside M) produced by Manus Bio demonstrates that the manufacturing process produces a consistent product which meets the established product specifications. A summary of the batch analyses is presented in Table B.3.1 and Table B.3.2

**Table B.3.1 Summary of the Product Analysis for 3 Non-Consecutive Lots of High-Purity Rebaudioside M (≥95% Rebaudioside M)**

| Specification Parameter | Limit | Manufacturing Lot No. | | |
|---|---|---|---|---|
| | | MAM060420 | MAM060520E | MAM061120A |
| Appearance | White to off-white powder | Pass | Pass | Pass |
| Total steviol glycosides (anhydrous basis) | ≥95% | >95 | >95 | >95 |
| Rebaudioside M | ≥95% | 97.93 | 97.01 | 97.2 |
| Loss on drying | ≤6% | 4.2 | 3.98 | 2.40 |
| pH (1% solution) | 4.5 to 7.0 | Pass | Pass | Pass |
| Residual ethanol | ≤5,000 ppm | Not Detected | Not Detected | Not Detected |
| Residual methanol | ≤200 ppm | Not Detected | Not Detected | Not Detected |
| Total ash | ≤1% | <1% | <1% | <1% |
| Lead | ≤1 ppm | <1 ppm | <1 ppm | <1 ppm |
| Arsenic | ≤1 ppm | <1 ppm | <1 ppm | <1 ppm |
| Cadmium | ≤1 ppm | <1 ppm | <1 ppm | <1 ppm |
| Mercury | ≤1 ppm | <1 ppm | <1 ppm | <1 ppm |
| Total plate count | <1,000 CFU/g | 50 CFU/g | 70 CFU/g | 10 CFU/g |

***Table B.3.2 Summary of the Product Analysis for 3 Non-Consecutive Lots of High-Purity Rebaudioside M (≥95% Rebaudioside M)***

| Specification Parameter | Limit | Manufacturing Lot No. | | |
|---|---|---|---|---|
| | | MAM060420 | MAM060520E | MAM061120A |
| Mold | <100 CFU/g | <10 CFU/g | <10 CFU/g | <10 CFU/g |
| Yeast | <100 CFU/g | <10 CFU/g | <10 CFU/g | <50 CFU/g |
| Coliforms | <3 MPN/g | <3 MPN/g | <3 MPN/g | <3 MPN/g |
| *Escherichia coli* | Not detected | Not detected | Not detected | Not detected |
| *Salmonella* | Negative/25 g | Negative | Negative | Negative |

CFU = colony-forming units; MPN = most probable number; NA = not applicable; ppm = parts per million.

### B.3.2 Reb I

Analysis of 3 non-consecutive lots (A003, 002A, 002C) of high-purity Rebaudioside I (≥95 % Rebaudioside I) demonstrates that the manufacturing process produces a consistent product which meets the established product specifications. A summary of the batch analyses is presented in Table B.3.2a and microbial analysis in Table B.3.2b. Product analysis has confirmed that Manus Bio's steviol glycoside product is comprised of Rebaudioside I, other steviol glycosides and water.

**Table B.3.2a Summary of the Product Analysis for 3 Non-Consecutive Lots of High-Purity Rebaudioside I (≥95% Rebaudioside I)**

| Specification Parameter | Specification | Manufacturing Lot No. | | |
|---|---|---|---|---|
| | | A003 | 002A | 002C |
| Appearance | White-to-off-white powder | Pass | Pass | Pass |
| Total steviol glycosides (anhydrous basis) | ≥95% | Pass | Pass | Pass |
| Rebaudioside I | ≥95% | 97.5% | 97.9% | 97.8% |
| Loss on drying | ≤6% | 2.6 % | 3.8 % | 3.0 % |
| pH (1% solution) | 4.5 to 7.0 | Pass | Pass | Pass |
| Residual ethanol | ≤5,000 ppm | 100 ppm | 200 ppm | 1100 ppm |
| Residual methanol | ≤200 ppm | Not Detected | Not Detected | Not Detected |
| Total ash | ≤1% | 0.36% | <0.001% | <0.001% |
| Lead | ≤1 ppm | <0.1ppm | <0.1 ppm | <0.1 ppm |
| Arsenic | ≤1 ppm | <0.1 ppm | <0.1 ppm | <0.1 ppm |
| Cadmium | ≤1 ppm | <0.02 ppm | <0.02 ppm | <0.02 ppm |
| Mercury | ≤1 ppm | <0.01 ppm | <0.01 ppm | <0.01 ppm |
| Total plate count | <1,000 CFU/ g | 970 CFU/ g | <1 CFU/ g | 210 CFU/ g |

**Table B.3.2b Summary of the Product Analysis for 3 Non-Consecutive Lots of High-Purity Rebaudioside I (≥95 % Rebaudioside I)**

| Specification Parameter | Specification | Manufacturing Lot No. | | |
|---|---|---|---|---|
| | | A003 | 002A | 002C |
| **Mold** | <100 CFU/ g | <10 CFU/ g | <10 CFU/ g | <10 CFU/ g |
| **Yeast** | <100 CFU/ g | <10 CFU/ g | <10 CFU/ g | <10 CFU/ g |
| **Coliforms** | <10 CFU/ g | <10 CFU/g | <10 CFU/ g | <10 CFU/ g |
| **Escherichia coli** | Not detected | Not detected | Not detected | Not detected |
| **Salmonella** | Negative/ 25 g | Negative | Negative | Negative |

CFU/g = colony-forming units per gram

### 3.3 Stability Data

#### 3.3.1 Reb M

A number of scientific and authoritative bodies, including JECFA, the European Food Safety Authority (EFSA), and Food Standards Australia/New Zealand (FSANZ), have reviewed the stability of steviol glycosides. The stability of steviol glycosides is also discussed in several published studies (Chang and Cook, 1983; Kroyer, 1999; Oehme et al., 2017). At the 68th meeting, JECFA evaluated the stability of steviol glycosides under conditions mimicking their use in foods and noted that steviol glycosides do not undergo browning or caramelization when heated and are stable under elevated temperatures (JECFA, 2007). In addition, steviol glycosides (approximately 90 to 94% purity) are stable for at least 180 days when stored at temperatures up to 24°C and pH 2.0 to 4.0. However, at elevated temperatures (80°C), steviol glycoside solutions maintained in water and pH 4.0 and 3.0 for 8 hours showed 4 and 8% decomposition, respectively. At temperatures of 100°C, higher rates of decomposition were observed, with 10 and 40% decomposed at pH 4.0 and 3.0, respectively. These results indicate that the stability of steviol glycosides is pH- and temperature-dependent. Based on the available evidence, JECFA concluded that steviol glycosides are thermally and hydrolytically stable for use in foods and acidic beverages under normal processing and storage conditions (JECFA, 2007).

The U.S. FDA has reviewed the stability of high-purity Rebaudioside M preparations in previous GRAS notices (GRN812, GRN759, GRN745, GRN744, GRN512, GRN882, GRN780 and GRN 846) (U.S. FDA, 2018, 2018, 2018, 2018, 2014, 2019, 2018, 2019). There exists a number of studies on the stability of steviol glycosides, including stevioside, Rebaudioside A, and Rebaudioside M, under different storage conditions (e.g., in different forms, such as powder and solution, in acidic conditions, and various temperatures) in the publicly available scientific literature (Wood et al., 1955; Chang and Cook, 1983; Kinghorn, 2002; Merisant, 2008; Chaturvedula et al., 2013; Prakash et al., 2014). Ultimately, the results of these stability studies suggest that the stability of steviol glycosides is pH- and temperature-dependent, which are consistent with the conclusions of JECFA (2007). More recently, a study evaluating the structural stability of 3 commercial batches each of the dried stevia leaves, the first aqueous infusion of the ground stevia, and a high-purity stevia leaf extract (≥95% steviol glycosides) confirmed that the

processing steps do not chemically alter or modify the steviol glycoside content (Oehme et. al., 2017).

In addition to the stability studies within the scientific literature, storage stability studies on Rebaudioside M were discussed in GRN 512 and GRN 667. GRN 512 presented the results of a stability test on 1 batch of Rebpure™ RM95, which contains ≥95% Rebaudioside M (U.S. FDA, 2014). In this study, a sample of Rebpure™ RM95 was stored at 25±5°C and relative humidity of 60±5% for up to 8 weeks. The results of the study demonstrate that the Rebaudioside M and the total steviol glycoside content remained ≥95% over the course of the 8-week study period. GRN 667 presented the results of an accelerated storage stability study on Rebaudioside M (≥95% Rebaudioside M) when stored at 40±2°C and relative humidity of 75±5% for up to 6 months (U.S. FDA, 2016). Over the course of the accelerated stability study, Rebaudioside M was observed to be stable in that the Rebaudioside M content did not change over the 6-month period and remained ≥95% Rebaudioside M.

The results of these storage stability studies are consistent with the results of JECFA (2007) in that the stability of steviol glycosides, including Rebaudioside M, are thermally stable under normal storage conditions.

Furthermore, while the Rebaudioside M content of Manus Bio's high-purity Rebaudioside M and that of the preparations described in GRN 512, GRN 667, GRN 745, GRN 759, GRN 780, GRN 799, GRN 812, GRN 846, and GRN 882 are similar in all Rebaudioside M preparations (i.e., ≥85% to ≥95% total steviol glycosides), Rebaudioside M is expected to exhibit similar chemical stability to other closely related steviol glycosides (e.g. stevioside & Rebaudioside A) based on their chemical structure similarity. Therefore, it is anticipated that the results of the stability studies on the Rebaudioside M preparations and the results of the stability studies available in the publicly available scientific literature, can be extended to support the stability of Manus Bio's high-purity Rebaudioside M (≥95% Rebaudioside M).

Manus Bio is currently conducting an accelerated stability study on 1 batch of high-purity Rebaudioside M (≥95% Rebaudioside M) produced by biotransformation of steviol glycosides (Lot No. MAM0605520E). In this study, sample of approximately 25 g of high-purity Rebaudioside M (≥95% Rebaudioside M) are stored at 50°C in polypropylene jars with screw top lids and heat sealed to mimic commercial packaging. Total steviol glycosides and Reb M content

have been measured by HPLC at baseline and at 1 and 3 months. The moisture content was measured by gravimetric moisture balance.

Preliminary results at 6 months indicate no significant changes in Reb M or steviol glycosides content (Table 3.3.1).

**Table 3.3.1 Results of an Accelerated Stability Study on 1 Batch of High-Purity Rebaudioside M (≥95% Rebaudioside M) (Lot No MAM 060520E)**

| Parameter | Month | | | |
|---|---|---|---|---|
| | 0 (baseline) | 1 | 3 | 6 |
| Total steviol glycosides (%) (dry basis) | 98.49 | 98.49 | 97.27 | 98.75% |
| Rebaudioside M (%) (dry basis) | 97.01 | 97.01 | 96.79 | 96.26% |

### 3.3.2 Reb I

A number of scientific and authoritative bodies, including JECFA, the European Food Safety Authority (EFSA), and Food Standards Australia/New Zealand (FSANZ), have reviewed the stability of steviol glycosides. The stability of steviol glycosides is also discussed in several published studies (Chang and Cook, 1983; Kroyer, 1999; Oehme et al., 2017). At the 68th meeting, JECFA evaluated the stability of steviol glycosides under conditions mimicking their use in foods and noted that steviol glycosides do not undergo browning or caramelization when heated and are stable under elevated temperatures (JECFA, 2007). In addition, steviol glycosides (approximately 90 to 94% purity) are stable for at least 180 days when stored at temperatures up to 24°C and pH 2.0 to 4.0. However, at elevated temperatures (80°C), steviol glycoside solutions maintained in water and pH 4.0 and 3.0 for 8 hours showed 4 and 8% decomposition, respectively. At temperatures of 100°C, higher rates of decomposition were observed, with 10 and 40% decomposed at pH 4.0 and 3.0, respectively. These results indicate that the stability of steviol glycosides is pH- and temperature-dependent. Based on the available evidence, JECFA concluded that steviol glycosides are thermally and hydrolytically

stable for use in foods and acidic beverages under normal processing and storage conditions (JECFA, 2007).

The U.S. FDA has reviewed the stability of high-purity Rebaudioside I preparations in a previous GRAS notice (GRN911) (U.S. FDA, 2021). There exists a number of studies on the stability of steviol glycosides, including stevioside, Rebaudioside A, and Rebaudioside M, Rebaudioside I under different storage conditions (e.g., in different forms, such as powder and solution, in acidic conditions, and various temperatures) and in the publicly available scientific literature (Wood et al., 1955; Chang and Cook, 1983; Kinghorn, 2002; Merisant, 2008; Chaturvedula et al., 2013; Prakash et al., 2014). These studies are discussed in detail in GRN512, GRN667, GRN780, and GRN846 and are incorporated by reference in this notice. Ultimately, the results of these stability studies suggest that the stability of steviol glycosides is pH- and temperature-dependent, which are consistent with the conclusions of JECFA (2007). More recently, a study evaluating the structural stability of 3 commercial batches each of the dried stevia leaves, the first aqueous infusion of the ground stevia, and a high-purity stevia leaf extract (~95% steviol glycosides) confirmed that the processing steps do not chemically alter or modify the steviol glycoside content (Oehmeet al., 2017).

In addition to the stability studies within the scientific literature, a storage stability studies on Rebaudioside I was discussed in GRN 911 in which the Rebaudioside I content did not change over the 6-month period and remained ≥95% Rebaudioside I. The results of these storage stability studies are consistent with the results reviewed by JECFA (2007) in that the stability of steviol glycosides, including Rebaudioside I, are thermally stable under normal storage conditions.

Rebaudioside I is expected to exhibit similar chemical stability to other closely related steviol glycosides (e.g. stevioside & Rebaudioside A) based on their chemical structure similarity. Therefore, it is anticipated that the results of the stability studies in the preparations described in GRN 512, GRN 667, GRN 745, GRN 759, GRN 780, GRN 799, GRN 812, GRN 846, GRN 911, and GRN 882 and the results of the stability studies available in the publicly available scientific literature, can be extended to support the stability of Manus Bio' s high-purity Rebaudioside I (≥95% Rebaudioside I).

Manus Bio has conducted a stability study on 2 batches of high purity Rebaudioside I (≥95% Rebaudioside I) produced by enzymatic conversion of steviol glycosides (Lot No. A003 and

002A). In this study, samples of approximately 25 g of high purity Rebaudioside I (≥95% Rebaudioside I) are stored at 25°C in polypropylene containers mimicking commercial packaging. Total steviol glycosides and Rebaudioside I content have been measured by HPLC at Time = 0, 3 and 6 months. Results demonstrate no significant changes in Rebaudioside I or steviol glycosides content (Table 3.3.2).

**Table 3.3.2 Results: Stability Study on 2 Batches of High-Purity Rebaudioside I (≥95% Rebaudioside I)**

| Lot No A003 (MAI2110281) | | | |
|---|---|---|---|
| Parameter | T= 0 | Month 3 | Month 6 |
| Loss on Drying (%) | 2.6 | 4.5 | 5.7 |
| Rebaudioside I (%) (HPLC) | 97.5 | 98.9 | 98.6 |

| Lot No 002A (MAI2110261) | | | |
|---|---|---|---|
| Parameter | T= 0 | Month 3 | Month 6 |
| Loss on Drying (%) | 3.8 | 5.4 | 5.8 |
| Rebaudioside I (%) (HPLC) | 97.8 | 98.1 | 97.9 |

# Appendix C

## 2.1 Statutory Declaration – Australia

Statutory Declarations Act 1959 [1]

I, Brendan Naulty based in 1762 Lovers Lane, Augusta, GA 30901, United States, Chief Commercial Officer of Manus Bio make the following declaration under the Statutory Declarations Act 1959:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the Statutory Declarations Act 1959, and I believe that the statements in this declaration are true in every particular.

Brendan Naulty (Dec 29, 2022 13:14 EST)

**Brendan Naulty**

Declared at 1762 Lovers Lane, Augusta, GA 30901, United States on 1 January 2023

Before me,

Sandra L. Ellis

Sandra L. Ellis (Jan 3, 2023 10:08 EST)

**Sandra L Ellis**

Public Notary, State of Georgia

1762 Lovers Lane, Augusta, GA 30901, United States

[1] http://www.comlaw.gov.au/Series/C1959A00052.

[2] A statutory declaration must be made before a prescribed person under the Statutory Declarations Act 1959.
The list of prescribed persons is available in the Statutory Declarations Regulations 1993 at http://www.comlaw.gov.au/Series/F1996B00198.

## 2.2 Statutory Declaration – New Zealand

Oaths and Declarations Act 1957[3]

I, Brendan Naulty of 1762 Lovers Lane Augusta, GA 30901, United States, Chief Commercial Officer of Manus Bio, solemnly and sincerely declare that:

1. the information provided in this application fully sets out the matters required; and
2. the information is true to the best of my knowledge and belief; and
3. no information has been withheld which might prejudice this application to the best of my knowledge and belief.

And I make this solemn declaration conscientiously believing the same to be true and by virtue of the Oaths and Declarations Act 1957

Brendan Naulty (Dec 29, 2022 13:14 EST)

**Brendan Naulty**

Declared at 1762 Lovers Lane Augusta, GA 30901, United States this 1 January 2023

**Declared before me**

Sandra L. Ellis

Sandra L. Ellis (Jan 3, 2023 10:08 EST)

**Sandra L Ellis**

Public Notary, State of Georgia

1762 Lovers Lane, Augusta, GA 30901, United States

[3] http://www.legislation.govt.nz/act/public/1957/0088/latest/DLM314553.html.

[4] A statutory declaration must be made before a person authorised to take a statutory declaration under section 9 of the Oaths and Declarations Act 1957.

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# To Sign Appendix C: v18 FINAL_FSANZ Petition_1 Jan 2023

Final Audit Report 2023-01-03

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