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Standard Reference Material® 3384
Ground Asian Ginseng (*Panax ginseng* C.A. Meyer) Rhizome
CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended primarily for evaluation of analytical methods for the determination of ginsenosides and trace elements in ground Asian ginseng (*Panax ginseng* C.A. Meyer) rhizomes and similar matrices. SRM 3384 can also be used for quality assurance when assigning values to in-house reference materials. This SRM has been characterized for its DNA sequence.

Description: A unit of SRM 3384 consists of five packets, each containing approximately 3 g of ground Asian ginseng rhizome.

Certified Values: A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias and variability have been taken into account [1].

Certified mass fraction values for elements in SRM 3384, reported on a dry-mass basis, are provided in Table 1. Analyses for value assignment were performed at NIST and by collaborating laboratories. Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2,3]. The measurands in Table 1 are total mass fractions for each analyte reported and metrological traceability is to the International System of Units (SI) derived unit for chemical mass fraction expressed as milligrams per kilogram [4].

Table 1. Certified Mass Fraction Values for Elements in SRM 3384

	Mass Fraction (mg/kg)		
Arsenic (As)	0.385	±	0.068
Lead (Pb)	6.42	±	0.61

Non-Certified Values: Non-certified values are provided in Appendix A.

Additional Information: Additional information is provided in Appendices B through F.

Period of Validity: The certified values delivered by **SRM 3384** are valid within the measurement uncertainty specified until **30 September 2029**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM to the end of the period of validity. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Steven J. Choquette, Director
Office of Reference Materials

Safety: SRM 3384 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

Storage and Handling: The SRM should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened packet until required for use. For elemental analyses, the packet can be opened, test portions removed for analyses, and then the packet resealed until the material reaches its expiration date. For ginsenoside analyses, the packet can be opened for removal of test portions and resealed for one week after initial opening of packet.

Use: Before use, the contents of a packet of material should be mixed thoroughly. To relate analytical determinations to the certified values in this Certificate of Analysis, the following masses used for NIST analyses should be used as the minimum sample size to ensure valid results: 0.5 g for elements and 30 mg for ginsenosides (see "Source and Preparation" below). Test portions should be analyzed as received and results converted to a dry-mass basis. The moisture conversion factor given below (see "Determination of Moisture") can be used for the sample(s) when using an unopened packet for the first time. If using a previously opened and resealed packet, moisture must be determined using one of the recommended techniques described below. Analytical results should include their own estimates of uncertainty and can be compared to the reference values using procedures described in reference 5.

Determination of Moisture: Moisture content of SRM 3384 was determined at NIST by (1) drying over magnesium perchlorate in a desiccator at room temperature for 35 d and (2) drying for 4 h in a forced-air oven at 90 °C. The means from both techniques were averaged to determine a dry-mass proportion of (0.9398 ± 0.0006) gram dry-mass per gram as-received mass; the uncertainty shown on this value is an expanded uncertainty to represent a 95 % level of confidence. The conversion factor used to convert data from an as-received to a dry-mass basis is the inverse of the dry-mass proportion. A relative uncertainty component of 0.03 % obtained from the moisture measurements is incorporated in the uncertainties of the assigned values, reported on a dry-mass basis, that are provided in this report.

Taxonomic Identification: The taxonomic identity is *Panax ginseng* established through identification by a trained botanist using an herbarium specimen from the original material and from associated DNA sequence analysis from botanically authenticated *Panax ginseng*. The associated DNA sequences are available in companion FASTA-formatted files [6]. The uncertainty associated with each nucleotide in the sequence, and in turn the uncertainty associated with the DNA sequence as an identifier of species, is expressed in an ordinal scale that represents the confidence estimates of the assigned value (Appendix B, Tables B1 and B2) [7]. These DNA sequences are used as a source of identity data for *Panax ginseng*.

Source and Preparation: The material for production of SRM 3384 was obtained through Modern Nutrition and Biotech (Ridgefield, CT). The material was received as nominally 180 μm (80 mesh) particle size and was packaged without additional grinding. The material was transferred to High-Purity Standards (Charleston, SC) where it was blended, aliquoted, and heat-sealed inside nitrogen-flushed 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel each. Following packaging, SRM 3384 was irradiated by Neutron Products, Inc. (Dickerson, MD) to an absorbed dose of 7.1 kGy to 8.5 kGy.

Homogeneity Assessment: The homogeneity of ginsenosides and elements was assessed at NIST using the methods and test portion sizes described below; analysis of variance with 5 % significance showed no evidence of inhomogeneity. The homogeneity of the DNA sequences was evaluated by sequencing the DNA from multiple samples from three randomly selected packets, revealing the degree of homogeneity in DNA sequence or species identity. The DNA data were homogeneous.

Value Assignment: For calculation of assigned values for analytes that were measured only by NIST, the mean of the mean values from NIST results was used. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, the weighted median of the laboratory means was used. For analytes that were also measured by NIST, the means of the individual sets of NIST data were averaged with the weighted median of the individual collaborating laboratory means, as appropriate.

Collaborating Laboratories' Analyses: The collaborating laboratories were asked to use their usual methods to make single measurements on test portions taken from each of three packets of SRM 3384. Because of the variability among data provided by laboratories participating in an interlaboratory comparison exercise, the weighted median of the individual laboratory means is used, and the uncertainty is estimated using a bootstrap procedure, both based on a Laplace random effects model [3,8,9].

Analytical Approach for Determination of Ginsenosides: Value assignment of the mass fractions of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 in SRM 3384 was based on measurements provided by NIST using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

NIST Analyses for Ginsenosides using LC-MS/MS: The mass fractions of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 were measured by LC-MS/MS in 30 mg test portions taken from each of ten packets of SRM 3384. Then 4-methylestradiol was added to each test portion as an internal standard and ginsenosides were extracted in equal portions of 60 % methanol/water and 60 % methanol/0.4 M potassium hydroxide by end-over-end rotation for 20 min followed by ultrasonication for 60 min. After centrifugation for 10 min at 3000 rpm, ginsenosides in the sample extracts were separated using a C18 column and monitored by tandem mass spectrometry in negative ion mode. The 4-methylestradiol internal standard was monitored by tandem mass spectrometry in positive ion mode. Calibrants were prepared from SRM 3389 *Ginsenosides Calibration Solutions* at levels intended to approximate the levels of the ginsenosides in the SRM following extraction. The purity of the neat calibrant materials used to prepare SRM 3389 was determined by NRC Canada using quantitative NMR (qNMR). A single internal standard solution was used for the calibrants and samples.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of elements in SRM 3384 was based on NIST results using inductively coupled plasma mass spectrometry (ICP-MS) and collaborating laboratories where appropriate.

NIST Analysis for As, Cd, and Pb Using ICP-MS: Mass fractions of arsenic, cadmium, and lead were determined by ICP-MS from duplicate, nominal 0.5 g test portions taken from each of four packets of the SRM. Test portions were digested in sealed vessels with a HNO_3/HF mixture using a microwave digestion system. Quantification was based on the method of standard additions using calibration solutions prepared from the SRM 3100 series of single-element standard solutions.

Methods Used for Certified Value Assignments: Methods used for certified value assignments are listed in Appendix A, Table A2.

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication (NIST SP) 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Oct 2021).

[2] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at https://www.bipm.org/en/publications/guides (accessed Oct 2021); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed Oct 2021).

[3] JCGM 101:2008; *Evaluation of Measurement Data — Supplement 1 to the "Guide to the Expression of Uncertainty in Measurement" — Propagation of Distributions Using a Monte Carlo Method*; Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/en/publications/guides (accessed Oct 2021).

[4] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/pml/special-publication-811 (accessed Oct 2021).

[5] Sharpless, K.E.; Duewer, D.L.; *Standard Reference Materials for Analysis of Dietary Supplements*; J. AOAC Int., Vol. 91, pp. 1298–1302 (2008).

[6] Reference data can be downloaded from the "Data and Information Files" link for the SRM available at https://www-s.nist.gov/srmors/view_detail.cfm?srm=3384 (accessed Oct 2021).

— The reference sequence data file for *rbcL* intron is:
SRM 3384 *trnL-trnF* sequence_v1 FASTA.docx

— The reference confidence estimates for every base of *rbcL-trnF* intron region sequence data file is:
SRM 3384 *trnL-trnF* Nucleotide Confidence_v1.rtf

— The reference sequence data file for ITS sequence is:
SRM 3384 ITS2 sequence_v1 FASTA.docx

— The reference confidence estimates for every base of ITS sequence data file is:
SRM 3384 ITS2 Nucleotide Confidence_v1.rtf

[7] SRM 2374; *DNA Sequence Library for External RNA Controls*; National Institute of Standards and Technology; U.S. Department of Commerce: Gaithersburg, MD (06 December 2017); available at https://www-s.nist.gov/srmors/view_detail.cfm?srm=2374 (accessed Oct 2021).
[8] Efron, B.; Tibshirani, R.J.; *An Introduction to the Bootstrap*; Chapman & Hall: London, UK (1993).
[9] Rukhin, A.L.; Possolo, A.; *Laplace Randon Effects Models for Interlaboratory Studies*; Computational Statistics and Data Analysis, Vol. 55, pp. 1815–1827 (2011).
[10] Sang, T.; Crawford, D.J.; Stuessy, T.F.; *Chloroplast DNA Phylogeny, Reticulate Evolution, and Biogeography of Paeonia (Paeoniaceae)*; Am. J. Bot., Vol. 84, pp. 1120–1136 (1997).
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[13] Labudde, R.A.; Harnly, J.M.; *Probability of Identification (POI): A Statistical Model for the Validation of Qualitative Botanical Identification Methods*; J. AOAC Int., Vol. 95, pp. 273–285 (2012).
[14] Wen, J.; Zimmer, E.A.; *Phylogeny and Biogeography of Panax L. (the Ginseng Genus, Araliaceae): Inferences from ITS Sequences of Nuclear Ribosomal DNA*; Molecular Phylogenetics and Evolution, Vol. 6, pp. 167–177 (1996).

Certain commercial equipment, instruments or materials may be identified in this Certificate of Analysis to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Non-Certified Mass Fraction Values: NIST non-certified values do not meet the NIST criteria for certification [1] and are the best estimates of the true values based on available data. The values are provided with an uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Non-certified mass fraction values for analytes in SRM 3384, reported on a dry-mass basis, are provided in Table A1 and are the mean of results provided by NIST measurements. Values are expressed as $x \pm U_{95\%}(x)$, where x is the non-certified value and $U_{95\%}(x)$ is the expanded uncertainty of the non-certified value. The method specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, the non-certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2,3]. The measurands in Table A1 are total mass fractions for each analyte reported and metrological traceability is to the SI derived unit for mass fraction [expressed as milligrams per kilogram (cadmium) or milligrams per gram (ginsenosides)] as realized by the methods used [4].

Table A1. Non-Certified Mass Fraction Values for Cadmium and Ginsenosides in SRM 3384

	Mass Fraction (mg/g)				Mass Fraction (mg/g)		
Ginsenoside Rb1	22.80	±	0.47	Ginsenoside Re	7.18	±	0.13
Ginsenoside Rb2	9.36	±	0.18	Ginsenoside Rf	1.354	±	0.022
Ginsenoside Rc	7.90	±	0.15	Ginsenoside Rg1	4.372	±	0.090
Ginsenoside Rd	4.700	±	0.093				

	Mass Fraction (mg/kg)		
Cadmium (Cd)	0.1970	±	0.0054

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **30 September 2029**. The value assignments are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the https://www.nist.gov/srm website.

Methods Used for Certified and Non-Certified Value Assignments: Methods used for certified and non-certified value assignments are listed in Table A2.

Table A2. Methods Used for Certified and Non-Certified Value Assignments

Element	Method
Arsenic (As)	ICP-MS, Collaborating Laboratories (ICP-MS, ICP-OES, AAS, TXRF)
Cadmium (Cd)	ICP-MS
Lead (Pb)	ICP-MS, Collaborating Laboratories (ICP-MS, ICP-OES, AAS)
Ginsenoside Rb1	LC-MS/MS
Ginsenoside Rb2	LC-MS/MS
Ginsenoside Rc	LC-MS/MS
Ginsenoside Rd	LC-MS/MS
Ginsenoside Re	LC-MS/MS
Ginsenoside Rf	LC-MS/MS
Ginsenoside Rg1	LC-MS/MS

AAS	Atomic absorption spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
LC-MS/MS	Liquid chromatology with tandem mass spectrometry
TXRF	Total reflection x-ray fluorescence spectroscopy

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APPENDIX B

Assignment of Identity

Molecular Approach for Species Identity: Sanger sequencing was used on two independent chloroplast gene regions, *trnL-trnF* intron [10–12], and the nuclear ribosomal gene internal transcribed spacer (ITS2) sequence for authentication of SRM 3384. Complete sequencing of the strands of DNA for the two gene regions was performed on triplicate samples, the sequence reads were independently hand-curated, and the confidence values were estimated as described below. DNA sequences are available in companion FASTA-formatted files [6]. To certify the species identity, validation studies were performed based on the "Probability of Identification (POI)" model using an Inclusivity and Exclusivity Panel [13]. Multiple samples of authenticated herbarium vouchers, botanical identity reference materials, and published literature were used to comprise the panels from the target and most closely related species (see Appendix C for more information on the reference samples). These samples were analyzed a minimum of ten times to ensure consistency in methods from replicate to replicate. Maximum Likelihood (ML) phylogenetic analyses were performed using the phylogenetic estimation using ML (PhyML) algorithm, with a General Time Reversible (GTR) substitution model, a fixed transition-transversion ratio, and 1,000 bootstrap replicates [8]. ML phylogenies, and the DNA aligned matrices, which can be found in Appendices D and E, were used to determine the species identity of SRM 3384 and to estimate the confidence (as described below). The confidence levels for both the *trnL-trnF* intron and the ITS2 sequence were "Most Confident (0)."

The taxonomic identification is *Panax ginseng* and the associated chloroplast DNA sequences from the *trnL-trnF* intron region and from the nuclear ribosomal gene internal transcribed spacer (ITS2) sequence are available in companion FASTA-formatted files [6]. The confidence associated with each nucleotide in the sequence, and in turn the confidence associated with the DNA sequence as an identifier of species, is expressed in an ordinal scale that represents the strength of the belief in the assigned value (Table B1) [7]. In the absence of a fully developed metrology for identity, these DNA sequences are used as a source of identity data for *Panax ginseng.* Chloroplast and nuclear ribosomal DNA sequences from botanically authenticated *Panax ginseng* samples are used to establish inclusivity; chloroplast and nuclear ribosomal DNA sequences from close relatives are used to establish exclusivity.

Nucleotide Identity: A set of heuristic, experience-based, rules (see Table B2) were used to establish confidence estimates for the nucleotides comprising the DNA sequences obtained from SRM 3384, and their use in identifying the species (see Table B1). The DNA sequences with curated confidence estimates are available in companion FASTA-formatted files [6]. The DNA aligned matrices used to determine the species identity and their confidence estimates are provided in Appendices D and E. The confidence associated with each nucleotide in the sequence in the FASTA files provided [6], and in turn the confidence associated with the DNA sequence as an identifier of species, is expressed in an ordinal scale that represents the strength of the belief in the assigned value (Table B2) [7]. Characteristics of sequence data and phylogenetic data used for species identity associated with the levels of the ordinal scale are described in Tables B1 and B2. The confidence estimates for the *trnL-trnF* intron region and ITS2 sequences are available in companion FASTA-formatted files [6].

Table B1. Definitions of Heuristic Rules for Confidence Estimates of Species Identity

Confidence Level	Species Identity
Most Confident (0)	Have very well-supported and well-resolved phylogeny and/or multiple diagnostic nucleotides differentiating species from closest relatives; have data from multiple samples of both an inclusivity and exclusivity panel; data from multiple independent gene regions agree.
Very Confident (1)	Have reasonably well-supported and well-resolved phylogeny and/or a few diagnostic nucleotides differentiating species from close relatives; have data from multiple samples of both an inclusivity and exclusivity panel; data from one gene, or data from multiple independent gene regions agree.
Confident (2)	Have reasonably well-supported and well-resolved phylogeny and/or one or a few diagnostic nucleotides differentiating species from close relatives; have data from a few samples of both an inclusivity and exclusivity panel; data from one gene, or data from multiple independent gene regions generally agree.
Ambiguous (3)	Have a poorly supported and poorly resolved phylogeny and/or no diagnostic nucleotides differentiating species from close relatives; have data from a few or multiple samples of both an inclusivity and exclusivity panel; data from one gene, or data from multiple independent gene regions generally disagree.

Table B2. Definitions of Heuristic Rules for Confidence Estimates of DNA Nucleotide Identity

Confidence Level	Nucleotide Identity
Most Confident (0)	Have good answers (fully reliable, unambiguous base calls) on both strands; all data from both strands agree.
Very Confident (1)	Have good answer on one strand; poor answer (less than fully reliable, potentially ambiguous base call) on the second/alternate strand; base calls from both strands typically agree, and there is biochemical context that explains the anomalous sequence data.
Confident (2)	Have good answer on one strand; anomalous sequence data that may give rise to a conflicting base call on the alternate strand; judgment required to resolve anomaly.
Ambiguous (3)	No clear mutually supporting results; unambiguous base calls disagree; or no unambiguous base calls on either strand; data from the two opposing strands could not be authoritatively reconciled.

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APPENDIX C

Reference Samples and Sequences Used in the Specificity Validation Study for SRM 3384

Table C1. Inclusivity Panel for *Panax ginseng* C.A. Meyer

Panax ginseng C.A. Meyer	Sample Source[a]	Voucher Information or GenBank Accession Number	ITS2[b], *trnL-trnF*[c]
1	AHP	AH072	+, +
2	AHP	2837	+, +
3	AHP	461	+, +
4	AHP	AH071	+, +
5	AHP	2709	+, +
6	AHP	433F	+, +
7	AHP	462	+, +
8	AHP	456	+, +
9	AHP	640	+, +

[a] AHP = American Herbal Pharmacopoeia (AHP – Verified Botanical Identity Reference Material).
[b] Internal Transcribed Spacer Sequence is included when a plus sign (+) is present, not included when a minus (–) is present.
[c] Intron sequence is included when a plus sign (+) is present, not included when a minus (–) is present.

Table C2. Exclusivity Panel for *Panax ginseng* C.A. Meyer[a]

Species	Sample Source[b] or Reference	Voucher Information or GenBank Accession Number	ITS2[c], *trnL-trnF*[d]
Panax quinquefolius	AHP	076	+, +
Panax notoginseng	AT	102	+, +
Panax trifolius	UC	874489	+, +
Panax japonicas	UC	986158	+, –
Withania somnifera	UC	1497411	+, +

[a] This table includes the examples of some common adulterants [14].
[b] AHP = American Herbal Pharmacopoeia (AHP – Verified Botanical Identity Reference Material); AT = AuthenTechnologies; UC = University Herbarium, University of California, Berkeley, CA.
[c] Internal Transcribed Spacer Sequence is included when a plus sign (+) is present, not included when a minus (–) is present.
[d] Intron sequence is included when a plus sign (+) is present, not included when a minus (–) is present.

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APPENDIX D

***trnL-trnF* DNA Aligned Matrix for *Panax ginseng* C.A. Meyer and Relatives**

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SRM 3384             GAAAACAAACAAAGGTTCAGAAGGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGC
Panax ginseng        GAAAACAAACAAAGGTTCAGAAGGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGC
Panax quinquefolius GAAAACAAACAAAGGTTCAGAAGGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGC
Panax notoginseng   GAAAACAAACAAAGGTTCAGAAGGCGA-AAAAGGGATAGGTGCAGAGACTCAATGGAAGC
Panax trifolius      GAAAACAAACAAAGGTTCAGAAGGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGC

SRM 3384             TGTTCTAACAAATGGAGTGGAATGTGTTGCATTGGTAGAGGAATCCTTCCATTGAAACTT
Panax ginseng        TGTTCTAACAAATGGAGTGGAATGTGTTGCATTGGTAGAGGAATCCTTCCATTGAAACTT
Panax quinquefolius TGTTCTAACAAATGGAGTGAAATGTGTTGCATTGGTAGAGGAATCCTTCCATTGAAACTT
Panax notoginseng   TGTTCTAACAAATGGAGTGGAATGTGTTGCATTGGTAGAGGAATCCTTCCATTGAAACTT
Panax trifolius      TGTTCTAACAAATGGAGTGGACTGTGTTGCATTGGTAGAGGAATCCTTCCATTGAAACTT

SRM 3384             CCAAAAGGATGAAGGATAAACGTATAGACATACGTATACGTACTGAAATACTCTATCAAA
Panax ginseng        CCAAAAGGATGAAGGATAAACGTATAGACATACGTATACGTACTGAAATACTCTATCAAA
Panax quinquefolius CCAAAAGGATGAAGGATAAACGTATAGACATACGTATACGTACTGAAATACTCTATCAAA
Panax notoginseng   CCAAAAGGATGAAGGATAAACGTATAGACATACGTATACGTACTGAAATACTCTATCAAA
Panax trifolius      CCAAAAGGATGAAGGATAAACGTATAGACATACGTATACGTACTGAAATACTCTATCAAA

SRM 3384             TGATTAATGACGACCCGAATCTCTATTTTTTATATGAAAACGGAAGAATTGTTGTGAATC
Panax ginseng        TGATTAATGACGACCCGAATCTCTATTTTTTATATGAAAACGGAAGAATTGTTGTGAATC
Panax quinquefolius TGATTAATGACGACCCGAATCTCTATTTTTTATATGAAAACGGAAGAATTGTTGTGAATC
Panax notoginseng   TGATTAATGACGACCCGAATCTCTATTTTTTATATGAAAACGGAAGAATTGTTGTGAATC
Panax trifolius      TGATTAATGACGACCCGAATCTGTATTTTTTATATGAAAACGGAAGAATTGTTGTGAATC

SRM 3384             GATTCCATATTGACGAAAGAATCGAATATTCATTGATCAAATAATTCACCCCATACATAG
Panax ginseng        GATTCCATATTGACGAAAGAATCGAATATTCATTGATCAAATAATTCACCCCATACATAG
Panax quinquefolius GATTCCATATTGACGAAAGAATCGAATATTCATTGATCAAATAATTCACCCCATACATAG
Panax notoginseng   GATTCCATATTGACGAAAGAATCGAATATTCATTGATCAAATAATTCACCCCATACATAG
Panax trifolius      GATTCCATATTGACGAAAGAATCGAATATTCATTGATCAAATCATTCACCCCATACATAG

SRM 3384             TCTGATAGTTCTTTTGAAGAACTGATTAATCGGACGAGAATAAAGATAGAGTCCCATTCT
Panax ginseng        TCTGATAGTTCTTTTGAAGAACTGATTAATCGGACGAGAATAAAGATAGAGTCCCATTCT
Panax quinquefolius TCTGATAGTTCTTTTGAAGAACTGATTAATCGGACGAGAATAAAGATAGAGTCCCATTCT
Panax notoginseng   TCTGATAGTTCTTTTGAAGAACTGATTAATCGGACGAGAATAAAGATAGAGTCCCATTCT
Panax trifolius      TCTGATAGTTCTTTTGAAAAACTAATGAATCGGACGAGAATAAAGATAGAGTCCCATTCT

SRM 3384             ACATGTCAATACCGGCAACAATGAAATTTTTAGTAAGAGGAAAATCCGTCGACTTTAAAA
Panax ginseng        ACATGTCAATACCGGCAACAATGAAATTTTTAGTAAGAGGAAAATCCGTCGACTTTAAAA
Panax quinquefolius ACATGTCAATACCGGCAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAAAA
Panax notoginseng   ACATGTCAATACCGGCAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAAAA
Panax trifolius      ACATGTCAATACCGGCAACAATGAAATTTATAGTAAGAGGAAAATCCGTCGACTTTAAAA

SRM 3384             TCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGCCCATTTGACTCCCTCATTTTTTATC
Panax ginseng        TCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGCCCATTTGACTCCCTCATTTTTTATC
Panax quinquefolius TCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGCCCATTTGACTCCCTCATTTTTTATC
Panax notoginseng   TCGTGAGGGTTCAAGTCCCTCTATCCCCAAAAAGCCCATTTGACTCCCTCATTTTTTATC
Panax trifolius      TCGTGAGGGTTCAAGTCCCTCTATCCCCAAAA-GCCCATTTGACTCCCTCATTTTTTATC

SRM 3384             CTATCCTTTTTCCGTTAGCAGTTAAAAATTCGTTATCTTTCTCATTCACCTACTCTTTTA
Panax ginseng        CTATCCTTTTTCCGTTAGCAGTTAAAAATTCGTTATCTTTCTCATTCACCTACTCTTTTA
Panax quinquefolius CTATCCTTTTTCCGTTAGCAGTTAAAAATTCGTTATCTTTCTCATTCACCTACTCTTTTA
Panax notoginseng   CTATCCTTTTTCCGTTAGCAGTTAAAAATCCGTTATCTTTCTCATTCACCTACTCTTTTA
Panax trifolius      CTATCCTTTTTCCGTTAGCAGTTAAAAATTCGTTATCTTTCTCATTCACCTACTCTTTTA

SRM 3384             CAAACGGATCTGAGCGGAAATATTTTTTCTCTTATCGCAGGTCTTGTGATATATGATACA
Panax ginseng        CAAACGGATCTGAGCGGAAATATTTTTTCTCTTATCGCAGGTCTTGTGATATATGATACA
Panax quinquefolius CAAACGGATCTGAGCGGAAATATTTTTTCTCTTATCGCAGGTCTTGTGATATATGATACA
Panax notoginseng   CAAACGGATCTGAGCGGGAATATTTTTTCTCTTATCGCAGGTCTTGTGATATATGATACA
Panax trifolius      CAAACGGATCTGAGCGTAAATATTTTTTCTCTTATCACAGGTCTTGTGATATATGATACA

SRM 3384             TGTACAAATGAACATCTTTGACCAAAGACTCCCCATTTGAATCAGTCACGGTCGATATCA
Panax ginseng        TGTACAAATGAACATCTTTGACCAAAGACTCCCCATTTGAATCAGTCACGGTCGATATCA
Panax quinquefolius TGTACAAATGAACATCTTTGACCAAAGACTCCCCATTTGAATCAGTCACGGTCGATATGA
Panax notoginseng   TGTACAAATGAACATCTTTGACCAAAGACTCCCCATTTGAATCAGTCACGGTCGATATCA
Panax trifolius      TGTACAAATGAACATCTTTGACCAAAGACTCCCCA---------GTCGCGGTCGATATCA
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SRM 3384             TTATTCATACTGAAACTTACAAAGTCTTCCCTTTTTGAAGATCCAAGAAATTCTAGGACC
Panax ginseng        TTATTCATACTGAAACTTACAAAGTCTTCCCTTTTTGAAGATCCAAGAAATTCTAGGACC
Panax quinquefolius TTATTCATACTGAAACTTACAAAGTCTTCCCTTTTTGAAGATCCAAGAAATTCTAGGACC
Panax notoginseng   TTATTCATACTGAAACTTACAAAGTCTTCCCTTTTTGAAGATCCAAGAAATTCTAGGACC
Panax trifolius     TTATTCATACTGAAACTTACAAAGTCTTCCCTTTTTGAAGATCCAAGAAATTCTAGGACC

SRM 3384             CGGATAAGACTTTGTAATACCCTTTCAATTGACATATAATTGA
Panax ginseng        CGGATAAGACTTTGTAATACCCTTTCAATTGACATATAATTGA
Panax quinquefolius CGGATAAGACTTTGTAATACCCTTCCAATTGACATATAATTGA
Panax notoginseng   CGGATAAGACTTTGTAATACCCTTTCAATTGA-----------
Panax trifolius     TGGATAAGACTTTGTAATACCCTTTCAATTGACATATAATTGA
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Figure D1. *trnL-trnF* DNA Aligned Matrix for *Panax ginseng* C.A. Meyer and Relatives. The results from the chloroplast region demonstrates that this region does distinguish this species from its relatives and does authenticate *Panax ginseng* C.A. Meyer. The four bases of DNA are represented as A = Adenine, T = Thymine, G = Guanine, and C = Cytosine, and "-" = missing data. The confidence estimate for the species identification of SRM 3384 as *Panax ginseng* C.A. Meyer is Most Confident (0).

* * * * * * * * * * * * End of Appendix D * * * * * * * * * * * *

APPENDIX E

ITS2 DNA Aligned Matrix for *Panax ginseng* C.A. Meyer and Relatives

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SRM 3384             ATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGC
Panax ginseng        ATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGC
Panax quinquefolius ATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGC
Panax notoginseng    ATCCCGTGAATCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGC
Panax trifolius      ATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGC
Panax japonicus      ATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGC

SRM 3384             ACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCAACCCATCACTCCCTTGCGGGAGT
Panax ginseng        ACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCAACCCATCACTCCCTTGCGGGAGT
Panax quinquefolius ACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCAACCCATCACTCCTTTGCGGGAGT
Panax notoginseng    ACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCAACCCATCATTCCCTCGCGGGAGT
Panax trifolius      ACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCAGCCCCGCACTCCCTCACCGGAGT
Panax japonicus      ACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCCAACCCATCACTCCCTTGCGGGAGT

SRM 3384             TGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAATGCGA
Panax ginseng        TGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAATGCGA
Panax quinquefolius CGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAATGCGA
Panax notoginseng    CGATGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGCGCGGTTGGCCCAAATGCGA
Panax trifolius      CGGGGCGGAGGGGCGGATACTGGCCTCCCGTGCCTAACCGCGCGGCTGGCCCAAATGCGA
Panax japonicus      CGAGGCGGAGGGGCGGATAATGGCCTCCCGTGTCTCACCGTGCGGTTGGCCCAAATGCGA

SRM 3384             GTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATGTCGT
Panax ginseng        GTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATGTCGT
Panax quinquefolius GTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATGTCGT
Panax notoginseng    GTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTTAAAAGCCCTCTTCTCATGTCGT
Panax trifolius      GTCCTCGGCGACGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCAAGTCGT
Panax japonicus      GTCCTTGGCGATGGACGTCACGACAAGTGGTGGTTGTAAAAAGCCCTCTTCTCATGTCGT

SRM 3384             GCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGTGCGC
Panax ginseng        GCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGTGCGC
Panax quinquefolius GCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGTGCGC
Panax notoginseng    GCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCTGTCCTCGACGCGCGC
Panax trifolius      GCGGTGACCCGTCGCCGGCAAAGGCTCTCACGACCCTGTTGCGCCGTCCCCGACGCGCGC
Panax japonicus      GCGGTGACCCGTCGCCAGCAAAAGCTCTCATGACCCTGTTGCGCCGTCCTCGACGTGCGC

SRM 3384             TCCGACCGCGACCCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCG
Panax ginseng        TCCGACCGCGACCCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCG
Panax quinquefolius TCCGACCGCGACCCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCG
Panax notoginseng    TCCGACCGCGACCCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCG
Panax trifolius      TCCGACCGCGACCCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCG
Panax japonicus      TCCGACCGCGACCCCAGGTCAGGCGGGACTACCCGCTGAGTTTAAGCATATCAATAAGCG

SRM 3384             GAGGAAAAGAAACTTACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAATAGCCCAG
Panax ginseng        GAGGAAAAGAAACTTACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAATAGCCCAG
Panax quinquefolius GAGGAAAAGAAACTTACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAATAGCCCAG
Panax notoginseng    GAGGAAAAGAAACTTACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAATAGCCCAG
Panax trifolius      GAGGAAAAGAAACTTACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAATAGCCCAG
Panax japonicus      GAGGAAAAGAAACTTACAAGGATTCCCCTAGTAACGGCGAGCGAACCGGGAATAGCCCAG

SRM 3384             CTTGAAAATCGGGCGACCCCG
Panax ginseng        CTTGAAAATCGGGCGACCCCG
Panax quinquefolius CTTGAAAATCGGGCGACCCCG
Panax notoginseng    CTTGAAAATTGGGCGACCTCG
Panax trifolius      CTTGAAAATCGGGCGACCTCG
Panax japonicus      CTTGAAAATCGGGCGACCCCG
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Figure E1. ITS2 DNA Aligned Matrix for *Panax ginseng* C.A. Meyer and Relatives. The results from the nuclear ribosomal gene region demonstrates that this region does distinguish this species from its relatives and is sufficient for identification of *Panax ginseng* C.A. Meyer. The four bases of DNA are represented as A = Adenine, T = Thymine, G = Guanine, and C = Cytosine. The confidence estimate for the species identification of SRM 3384 as *Panax ginseng* C.A. Meyer is Most Confident (0).

* * * * * * * * * * * * End of Appendix E * * * * * * * * * * * *

APPENDIX F

Collaborating Laboratories Contributing Data to Value Assignment of SRM 3384 and Responsibilities

Responsibilities
The development of SRM 3384 was a collaboration among the National Institute of Standards and Technology (NIST), the National Institutes of Health Office of Dietary Supplements (NIH-ODS), and the Food and Drug Administration Center for Drug Evaluation and Research (FDA CDER). The addition of genetic information was accomplished through collaboration among NIST, NIH-ODS, the U.S. Department of Agriculture Agricultural Research Service (USDA ARS), NSF International (formerly AuthenTechnologies, Richmond, CA), and American Herbal Pharmacopoeia (Scotts Valley, CA).

Coordination: C.A. Rimmer and L.J. Wood of the NIST Chemical Sciences Division.

Analytical Measurements: C.A. Barber, H.V. Hayes, and L.J. Wood of the NIST Chemical Sciences Division, and M.R. Ale, formerly of NIST.

Statistical Analysis: J.H. Yen of the NIST Statistical Engineering Division.

Institutional Support: Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Collaborating Laboratories
Analysts at the following laboratories performed measurements that contributed to the value assignment of toxic elements in SRM 3384 as part of an interlaboratory comparison exercise coordinated by NIST:

Advanced Botanical Consulting & Testing, Inc. (Tustin, CA)
Advanced Laboratories, Inc. (South Salt Lake City, UT)
ALS Environmental (Salt Lake City, UT)
Analytical Resource Labs (Lehi, UT)
Apex Analytical Laboratory (Tempe, AZ)
Arizona Nutritional Supplements (Chandler, AZ)
Brooks Applied Labs (Bothell, WA)
Covance Labs (Madison, WI)
Dyad Labs (Salt Lake City, UT)
Exova, Inc. (Santa Fe Springs, CA)
Gaia Herbs, Inc. (Brevard, NC)
HVL, LLC (Pittsburgh, PA)
Innovatical Laboratories, LLC (Montclair, CA)
International Vitamin Corporation (Mira Loma, CA)
Intertek Champaign Laboratories (Champaign, IL)
Natural Factors (Coquitlam, BC, Canada)
Natural Remedies Private Limited (Bangalore, Karnataka, India)
Nature's Way (Green Bay, WI)
NOW Foods (Bloomingdale, IL)
NSF International (Ann Arbor, MI)
Nutra Manufacturing (Greenville, SC)
Red Rock Laboratories, LLC (Tempe, AZ)
SGS Canada, Inc (Burnaby, BC, Canada)
Shree Dhootapapeshwar Ltd (Mumbai, India)
Underwriters Laboratories (Canton, MA)
US Food and Drug Admin CFSAN (GA) (Alemeda, GA)
US Food and Drug Admin CFSAN (MD) (College Park, MD)
US Food and Drug Admin CFSAN (NY) (Queens, NY)

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