NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

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23 January 2025

Standard Reference Material® 3234

Soy Flour

CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for validating methods for determining elements in soy flour and similar materials and can be used for quality assurance, such as when assigning values to in-house control materials.

Description: A unit of SRM 3234 consists of one bottle containing approximately 50 g of defatted soy flour prepared by a commercial manufacturer. The bottle is sealed inside an aluminized pouch.

Certified Values: NIST certified values are traceable to the International System of Units (SI) derived unit of mass fraction, expressed as milligrams per kilogram. The values are reported on a dry-mass basis [1].

	Mass Fraction[(a)] (mg/kg)
Calcium (Ca)	3191 ± 56
Copper (Cu)	15.34 ± 0.26
Iron (Fe)	80.3 ± 2.7
Magnesium (Mg)	3487 ± 60
Manganese (Mn)	36.78 ± 0.88
Phosphorus (P)	8080 ± 210
Potassium (K)	25010 ± 560
Zinc (Zn)	48.9 ± 1.1

[(a)] 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 $U_{95\%}(x)/2$ [2–4].

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

Additional Information: Additional information is provided in Appendices B and C.

Period of Validity: The certified values delivered by **SRM 3234** are valid within the measurement uncertainty specified until **20 August 2032**. 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 over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

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Safety: SRM 3234 IS INTENDED FOR RESEARCH USE; not for human consumption. Consult the Safety Data Sheet (SDS) for hazard information.

Storage: New unopened bottles of SRM 3234 should be stored at room temperature (20 °C ± 5 °C). Assigned elemental mass fractions values from a previously opened bottle are valid until the material reaches its expiration date, provided that the open bottle is resealed and stored at room temperature (20 °C ± 5 °C).

Use: Before use, the contents of the sealed bottle should be mixed thoroughly by rotating and/or rolling then allowed to settle for one minute prior to opening to minimize the loss of fine particles. Homogeneity of the material has not been evaluated for sample sizes smaller than those used by NIST methods described below. Therefore, the certified values may not be valid for test portions smaller than 0.5 g for determination of elements. Test portions should be analyzed as received and results converted to a dry-mass basis by determining moisture content on a separate test portion.

Source and Preparation: Three hundred twenty kilograms (from fourteen 50-pound bags) of defatted soy flour were blended and bottled by High-Purity Standards (Charleston, SC). The soy flour was placed in 4-ounce amber glass bottles that had been flushed with nitrogen. The bottles were capped and sealed with heat-shrink tape, then individually sealed in Mylar bags. Following bottling, SRM 3234 was irradiated by Neutron Products, Inc. (Dickerson, MD) to an absorbed dose of 7 kGy to 10 kGy.

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 Jan 2025).

[2] 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 Jan 2025).

[3] 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 (JCGM) (2008); available at https://www.bipm.org/en/publications/guides (accessed Jan 2025); 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 Jan 2025).

[4] 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*; JCGM (2008); available at https://www.bipm.org/en/publications/guides (accessed Jan 2025).

[5] Phillips, M.M.; Bedner, M.; Reitz, M.; Burdette, C.Q.; Nelson, M.A.; Yen, J.H.; Sander, L.C.; Rimmer, C.A.; *Liquid Chromatography with Absorbance Detection and with Isotope-Dilution Mass Spectrometry for Determination of Isoflavones in Soy Standard Reference Materials*; Analytical and Bioanalytical Chemistry, Vol. 409, pp. 949-960 (2016).

[6] Bryan Sallee, C.E.; Phillips, M.M.; Barber, C.A.; Burdette, C.Q., Kotoski, S.P.; Wood, L.J. (2023) *Food Nutrition and Safety Measurements Quality Assurance Program: Exercise 1 Final Report*. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Internal Report (IR) NIST IR 8447r1. Available at https://nvlpubs.nist.gov/nistpubs/ir/2023/NIST.IR.8447r1.pdf (accessed Jan 2025).

[7] Rukhin, A.L.; Possolo, A.; *Laplace Random Effects Models for Interlaboratory Studies;* Computational Statistics and Data Analysis, Vol. 55, pp. 1815–1827 (2011).

[8] Searle, S.R.; Casella, G.; McCulloch, C.L.; *Variance Components;* John Wiley; Hoboken, NJ (1992).

Certificate Revision History: 23 January 2025 (Addition of non-certified method-specific values for forms of dietary fiber to the table in Appendix A; editorial changes); **20 July 2022** (Correction to units in Table 1; editorial changes); **13 May 2022** (Change of period of validity; removal of certified values for riboflavin and pantothenic acid based on potential instability and NIST's decision to no longer support these measurement capabilities in this matrix; correction of name of cysteine to cystine; updated format; editorial changes); **25 October 2017** (Change of expiration date; removal of certified values for thiamine, niacin, niacinamide, total vitamin B_3, pyridoxal hydrochloride, pyridoxamine dihydrochloride, pyridoxine hydrochloride, and total vitamin B_6 based on NIST's decision to no longer support these measurement capabilities in this matrix; removal of certified values for choline and carnitine based on observed instability; removal of reference values for fatty acids based on observed instability; correction of value for phenylalanine; editorial changes); **15 October 2014** (Addition of reference values for isoflavones; removal of reference value for solids; editorial changes); **08 July 2013** (Addition of certified values for forms of vitamin B_6; editorial changes); **28 September 2012** (Original certificate date).

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 in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

* * * * * * * * * * * * End of Certificate of Analysis * * * * * * * * * * * *

APPENDIX A

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not meet the NIST criteria for certification [1] nor provide metrological traceability to the International System of Units (SI). They 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. Information about methods used to determine non-certified values is summarized in Appendix B.

Non-Certified Values (Dry-Mass Basis) for Additional Analytes in SRM 3234

	Mass Fraction[a] (mg/kg)
Sodium (Na)	2.52 ± 0.45
Daidzein	14.0 ± 3.0
Daidzin[c]	1680 ± 530
Genistein	15.49 ± 0.30
Genistin[c]	2080 ± 520
Glycitin[c]	245 ± 46

	Mass Fraction[a] (g/100 g)	n[b]
Ash	6.77 ± 0.14	14
Protein	53.37 ± 0.36	14
Fat (sum of fatty acids as triglycerides)	1.49 ± 0.12	13
Carbohydrates	37.14 ± 0.69	14
Total Dietary Fiber	18.19 ± 0.37	10
Total Dietary Fiber (AOAC 991.43)	18.4 ± 2.6	7
Total Dietary Fiber (AOAC 2017.16)	30.3 ± 6.6	2
Insoluble Dietary Fiber (AOAC 985.29/991.43)	16.2 ± 3.1	4
Soluble Dietary Fiber (AOAC 991.43)	2.9 ± 1.6	2
SDFS[d] (AOAC 2017.16)	7.28 ± 0.70	2
SDFP[d] (AOAC 2017.16)	4.0 ± 1.8	2
High Molecular Weight Dietary Fiber (AOAC 2017.16)	23.0 ± 7.2	2

	Mass Fraction[a] (g/100 g)	n[b]
Alanine	2.28 ± 0.16	6
Arginine	3.72 ± 0.31	6
Aspartic Acid	6.0 ± 1.2	6
Cystine	0.74 ± 0.15	4
Glutamic Acid	10.2 ± 1.4	6
Glycine	2.22 ± 0.15	6
Histidine	1.222 ± 0.089	6
Isoleucine	2.31 ± 0.23	6
Leucine	4.03 ± 0.42	6
Lysine	3.20 ± 0.25	6
Methionine	0.69 ± 0.13	6
Phenylalanine	2.54 ± 0.13	6
Proline	2.71 ± 0.23	6
Serine	2.69 ± 0.32	6
Threonine	2.02 ± 0.11	6
Tryptophan	0.66 ± 0.14	4
Tyrosine	1.76 ± 0.43	6
Valine	2.45 ± 0.41	6

	Mass Fraction[a] (kcal/100 g)	n[b]
Calories	377.7 ± 3.7	13

[a] These 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. To propagate this uncertainty, treat the non-certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–4].

[b] Number of contributors providing technically valid results.

[c] Value was determined using a hydrolysis approach, and therefore represents total glycosides (sum of glycoside, malonyl-glycoside, and acetyl-glycoside forms present in the material).

[d] SDFS: Soluble dietary fiber which remains soluble in 78 % aqueous ethanol; SDFP: Soluble dietary fiber that precipitates in 78 % aqueous ethanol

Period of Validity: The non-certified values are valid within the measurement uncertainty specified until **20 August 2032**. 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 and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

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APPENDIX B

Methods Used in Value Assignment of SRM 3234

Elements: Value assignment of the mass fractions of the elements in SRM 3234 was based on the combination of results from measurements made by NIST and collaborating laboratories, where available, as described below.

Methods Used in Value Assignment for Elements in SRM 3234[a,b]

Element	NIST Method(s)	Method(s) Reported by Collaborating Laboratories
Calcium (Ca)	ICP-OES	ICP-OES
Copper (Cu)	ICP-OES	ICP-OES
Iron (Fe)	ICP-OES	ICP-OES
Magnesium (Mg)	ICP-OES	ICP-OES
Manganese (Mn)	ICP-OES	ICP-OES
Phosphorus (P)	ICP-OES	ICP-OES, absorption spectrophotometry
Potassium (K)	ICP-OES	ICP-OES
Sodium (Na)	ICP-OES	ICP-OES
Zinc (Zn)	ICP-OES	ICP-OES

[a] ICP-OES: inductively coupled plasma optical emission spectrometry
[b] Not all collaborating laboratories reported methods used.

NIST Analyses for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn Using ICP-OES: The mass fractions of calcium, copper, iron, potassium, magnesium, manganese, phosphorus, and zinc were measured by ICP-OES using duplicate 0.5 g test portions taken from each of 12 bottles of SRM 3234. The mass fraction of sodium was measured by ICP-OES using duplicate 1.0 g test portions taken from each of 12 bottles of SRM 3234. Samples for ICP-OES were digested in a nitric acid/hydrofluoric acid mixture using a microwave sample preparation system. Indium was added as an internal standard, and quantification for all elements was based on the method of standard additions using the SRM 3100 series single element standard solutions.

Isoflavones: Value assignment of the mass fractions of isoflavones in SRM 3234 was based on the results from measurements made by NIST.

NIST Analyses for Isoflavones Using LC-absorbance: Mass fractions of daidzein, daidzin, genistein, genistin, and glycitin were measured by LC-absorbance in duplicate 200 mg test portions taken from each of 12 bottles of SRM 3234 [5]. A typical chromatogram is provided in Figure B1.

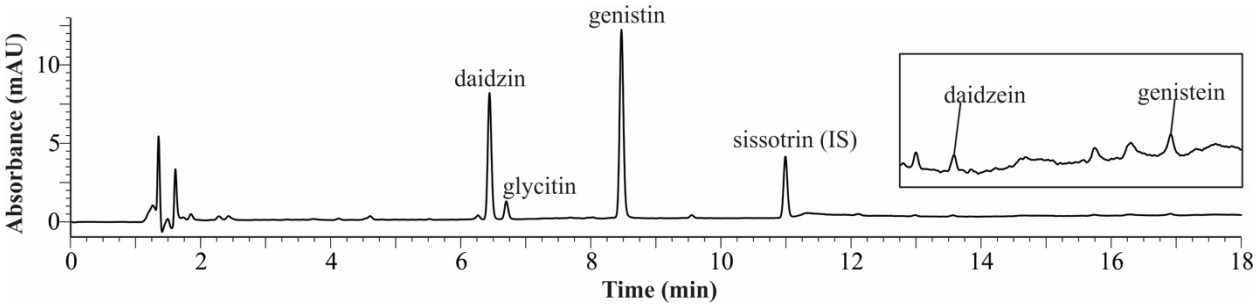

Figure B1. Chromatogram showing separation and detection of isoflavones in SRM 3234 using LC-absorbance [5].

NIST Analyses for Isoflavones Using ID-LC-MS: The mass fractions of daidzein, daidzin, genistein, genistin, and glycitin were measured by ID-LC-MS in duplicate 100 mg test portions taken from each of 12 bottles of SRM 3234 [5]. A typical chromatogram is provided in Figure B2.

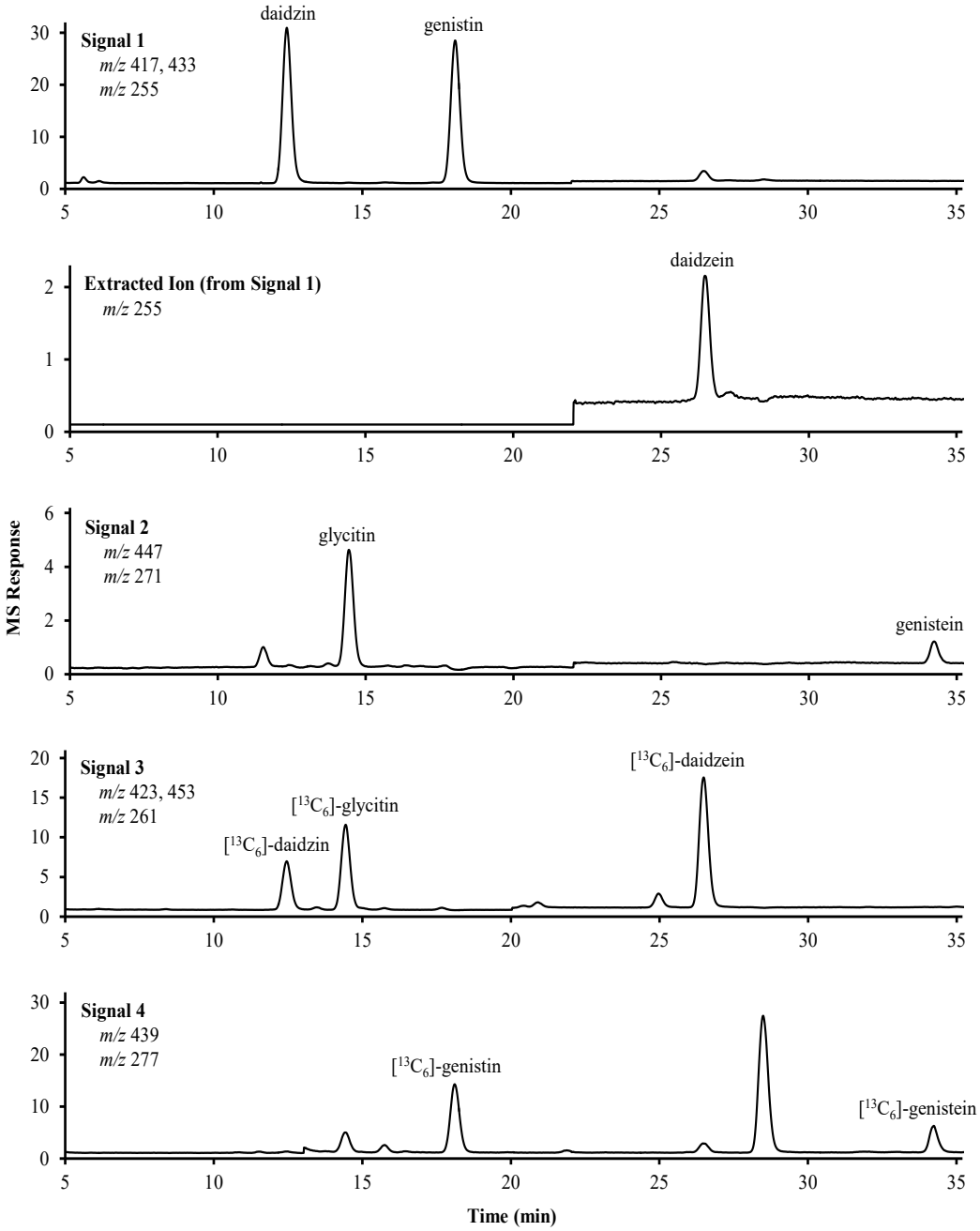

Figure B2. Chromatograms showing separation and detection of isoflavones in SRM 3234 using ID-LC-MS [5].

Amino Acids: Value assignment of the mass fractions of amino acids in SRM 3234 was based on the combination of measurements made by collaborating laboratories. Collaborating laboratories reporting amino acids data used acid digestion and hydrolysis followed by liquid chromatography with absorbance detection.

Proximates, Dietary Fiber, and Calories: Value assignment of the mass fractions of remaining measurands in SRM 3234 was based on the combination of results from measurements made by collaborating laboratories, as described in Table B2.

Table B2. Methods Used in Value Assignment for Additional Measurands in SRM 3234

Analyte	Method(s) Reported by Collaborating Laboratories[(a)]
Ash	Weight loss after ignition in a muffle furnace
	Thermogravimetric analysis
Fat	Sum of fatty acids by gas chromatography
Protein	Kjeldahl (nitrogen results converted to protein using a factor of 6.25)
	Thermal conductivity (nitrogen results converted to protein using a factor of 6.25)
	Pyrolysis gas chromatography (nitrogen results converted to protein using a factor of 6.25)
Carbohydrates	Calculation as [solids – (protein + fat + ash)]
Calories	Calculation as [(9 x fat) + (4 x protein) + (4 x carbohydrate)]
Dietary Fiber	Enzymatic digestion and gravimetry (AOAC 985.29, AOAC 991.43, AOAC 2017.16)

[(a)] Not all laboratories reported methods used.

Collaborating Laboratories' Analyses: The collaborating laboratories from Grocery Manufacturers Association (GMA) Food Industry Analytical Chemists Committee (FIACC) were asked to use their usual methods to make duplicate measurements on test portions taken from each of two samples of SRM 3234. Collaborating laboratories from the NIST Food Nutrition and Safety Measurements Quality Assurance Program (FNSQAP) Exercise 1 were asked to use their usual methods to make single measurements on test portions taken from each of three samples of SRM 3234 [6]. 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 based on a Laplace random effects model [7]. The uncertainty is estimated using a bootstrap procedure based on Laplace random effects model for the between-laboratory and within-laboratory effects [4,7,8]. Collaborating laboratories' data alone were used to assign reference values for proximates, calories, dietary fiber, and amino acids.

Homogeneity Assessment: The homogeneity of elements was assessed at NIST using the method and test portion size described. Analysis of variance did not show statistically significant heterogeneity. Other analytes have been treated as though they are homogeneously distributed in the material.

Value Assignment: For calculation of assigned values for analytes that were measured only by NIST, the mean of the NIST results was used. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, the median of the laboratory means was used. For analytes that were measured by both NIST and collaborating laboratories, the mean of the NIST data and the median of the individual collaborating laboratory means were averaged, as appropriate. All assigned values were determined on an as-received basis and converted to a dry-mass basis using a conversion factor that is the inverse of the dry-mass proportion. The dry-mass-proportion of (0.9387 ± 0.0049) gram dry mass per gram as-received mass was determined by averaging results obtained at NIST by using (1) freeze drying to constant mass over 7 d; (2) drying over magnesium perchlorate in a desiccator at room temperature for 21 d; and (3) drying in a forced-air oven at 90 °C for 2 h. The uncertainty in the dry mass-proportion is an expanded uncertainty ($k = 2$) corresponding to a 95 % level of confidence. An uncertainty component for the conversion factor (0.26 %) obtained from the moisture measurements is incorporated in the uncertainties of the certified and non-certified values, reported on a dry-mass basis, that are provided in this certificate and its appendices.

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APPENDIX C

Contributors to the Development and Value Assignment of SRM 3234

Coordination of Development, Production, and Technical Measurements
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Collaborating Laboratories
Grocery Manufacturers Association (GMA) Food Industry Analytical Chemists Committee (FIACC)
- Campbell Soup Company (Camden, NJ, USA)
- Conagra Foods (Omaha, NE, USA)
- Covance Laboratories, Inc. (Madison, WI, USA)
- Del Monte Foods (Walnut Creek, CA, USA)
- Eurofins Central Analytical Laboratories (Metairie, LA, USA)
- Eurofins Scientific (Des Moines, IA, USA)
- General Mills, Inc. (Golden Valley, MN, USA)
- Hormel Foods Corporation (Austin, MN, USA)
- Krueger Food Laboratories (Billerica, MA, USA)
- Land O'Lakes (Arden Hills, MN, USA)
- Schwan Food Company (Salina, KS, USA)
- Silliker (Crete, IL, USA)
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