National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3233

Fortified Breakfast Cereal

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining proximates, sugars, dietary fiber, vitamins, elements, and amino acids in fortified breakfast cereals and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a wheat-based fortified breakfast cereal prepared by a commercial manufacturer. A unit of SRM 3233 consists of one bottle containing approximately 60 g of material and sealed inside an aluminized pouch.

Certified Mass Fraction Values: The certified mass fraction values of selected elements and vitamins in SRM 3233 are provided in Tables 1 and 2, respectively. 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 have been investigated or taken into account [1]. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified values were calculated as the mean of the mean values from NIST methods, the mean from U.S. Department of Agriculture (USDA) methods, and the median of the mean results provided by collaborating laboratories, where appropriate. All values were combined without weighting. The associated uncertainties are expressed at the 95 % level of confidence [2–4]. Values are reported on a dry-mass basis in mass fraction units [5].

Reference Mass Fraction Values: Reference mass fraction values are provided for additional elements (Table 3), vitamins (Table 4), proximates, sugars, and calories (Table 5), dietary fiber (Table 6), and amino acids (Table 7). A NIST reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification [1] and is provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The reference mass fraction values were derived from results reported by NIST or collaborating laboratories. Values are reported on a dry-mass basis in mass fraction units [5].

Information Mass Fraction Values: Information mass fraction values for several elements determined using a single method at NIST are provided in Table 8. Information mass fraction values for several forms of dietary fiber determined by a single collaborating laboratory are provided in Table 9. A NIST information value is a value that may be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value, therefore no uncertainty is provided. Values are reported on a dry-mass basis in mass fraction units [5].

Expiration of Certification: The certification of **SRM 3233** is valid, within the measurement uncertainty specified, until **20 June 2017**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by K.E. Sharpless and L.J. Wood of the NIST Chemical Sciences Division and S. Ehling of the Grocery Manufacturers Association (GMA, Washington, DC).

Carlos A. Gonzalez, Chief
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Robert L. Watters, Jr., Director
Office of Reference Materials

Analytical measurements at NIST were performed by C. Bryan, J. Camara, S.K.R. Chinthalapati, W.C. Davis, L. Francini, J.L. Molloy, I.O. Mugenya, K.E. Murphy, Y. Nuevo Ordóñez, R. Oflaz, D.J. O'Kelly, T.O. Okumu, R.L. Paul, M.M. Phillips, B.J. Porter, C.A. Rimmer, J.B. Thomas, B.E. Tomlin, T.W. Vetter, L.J. Wood, and L.L. Yu of the NIST Chemical Sciences Division. Analyses for value assignment were also performed by R. Goldschmidt and W.R. Wolf of the Food Composition Methods Development Laboratory, Agricultural Research Service, USDA (Beltsville, MD), and the following laboratories participating in a GMA Food Industry Analytical Chemists Committee's (FIACC's) interlaboratory comparison exercise: Campbell Soup Company, Camden, NJ; Conagra Foods, Omaha, NE; Covance, Inc., Madison, WI; Del Monte Foods, Walnut Creek, CA; Eurofins Central Analytical Laboratories, Metairie, LA; Eurofins Scientific, Des Moines, IA; General Mills, Inc., Golden Valley, MN; Hormel Foods Corporation, Austin, MN; Krueger Food Laboratories, Billerica, MA; Land O'Lakes, Arden Hills, MN; Schwan Food Company, Salina, KS; Silliker, Madison, WI; The J.M. Smucker Co., Orville, OH; The National Food Laboratory, Livermore, CA. Five of these laboratories measured sugars: Campbell Soup Company; Covance, Inc.; Eurofins Central Analytical Laboratories; Hormel Foods Corporation; and Krueger Food Laboratories. Four of the laboratories plus one other measured dietary fiber: Covance, Inc.; Eurofins Central Analytical Laboratories; General Mills, Inc.; Megazyme International Ireland Ltd., Bray, County Wicklow, Ireland; and Silliker.

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

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

NOTICE TO USERS: SRM 3233 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened bottles. For elemental analyses, the bottle can be re-capped and test portions removed and analyzed until the material reaches its expiration date. For vitamin analyses, the bottle can be re-capped and test portions removed and analyzed for several months after the bottle was first opened. Water-soluble vitamins are stable in previously opened and tightly recapped bottles for at least one year when stored at room temperature or under refrigeration (4 °C).

Use: Before use, the contents of the bottle should be mixed thoroughly by rotating and/or rolling. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. For certified values to be valid, test portions of the following masses should be used: between 0.3 g and 0.5 g for elemental analysis and between 0.5 g and 10 g for vitamin analysis. Test portions should be analyzed as received and results converted to a dry-mass basis by determining moisture content (using one of the methods described below) on a separate test portion. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 6.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: The SRM is a fortified breakfast cereal. Two hundred kilograms (450 lbs) of fortified breakfast cereal was received as flakes in a single large box. The contents of the box were ground to 180 μm (80 mesh), blended, and bottled by High-Purity Standards (Charleston, SC). The cereal powder was placed in 4 oz amber bottles that had been flushed with nitrogen. Each bottle contains 60 g of cereal powder. The bottles were capped and sealed with heat-shrink tape, then individually sealed in Mylar bags. Following bottling, SRM 3233 was irradiated by Neutron Products, Inc. (Dickerson, MD) to an absorbed dose of 9.0 kGy to 11.5 kGy.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 3233 was based on the combination of measurements from two different analytical methods at NIST and collaborating laboratories, where available. NIST provided measurements by using inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS), instrumental neutron activation analysis (INAA), and radiochemical neutron activation analysis (RNAA).

[(1)] Certain commercial equipment, instruments or materials are identified in this certificate 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.

NIST Analyses for Ba, Ca, Cr, Co, Cu, Fe, I, K, Mg, Mn, Mo, Na, Ni, P, Sn, Sr, V, and Zn Using ICP-OES and/or ICP-MS: Barium, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, strontium, and zinc were measured by ICP-OES using duplicate 0.5 g test portions taken from each of 10 bottles of SRM 3233. Samples for ICP-OES were digested in a nitric acid/hydrofluoric acid mixture using a microwave sample preparation system. Barium, chromium, cobalt, molybdenum, nickel, strontium, tin, and vanadium were measured by ICP-MS in duplicate 0.25 g test portions taken from each of 10 bottles. Samples were digested in nitric acid using a microwave sample preparation system. Iodine was measured by ICP-MS in single 0.3 g test portions taken from each of six bottles. Samples were digested in aqueous tetramethylammonium hydroxide using a microwave sample preparation system. Quantitation for ICP-OES and ICP-MS was based on the method of standard additions. Tin was not homogeneously distributed in SRM 3233, with values ranging between 0.04 μg/g and 0.3 μg/g, and a value for tin could not be assigned (see "Homogeneity Assessment"). Similarly, values for chromium ranged from 3 μg/g to 5 μg/g, values for nickel ranged from 2 μg/g to 4 μg/g, and values were not assigned.

NIST Analyses for Cd and Pb Using Isotope Dilution ICP-MS: Cadmium and lead were measured by ID-ICP-MS using duplicate 0.5 g test portions taken from each of six bottles of SRM 3233. Samples were spiked with ^{111}Cd and ^{206}Pb and were digested in nitric acid using a microwave sample preparation system. Sample digests were evaporated to near dryness and a portion was reconstituted in dilute nitric acid for Pb analysis. To remove spectral interferences in the cadmium determination, the remaining portions of the sample digests were evaporated to dryness and reconstituted in water and concentrated hydrochloric acid to convert the nitrate form to the chloride. Samples were evaporated to dryness and again reconstituted in concentrated hydrochloric acid, which was again evaporated. Salts were dissolved in hydrochloric and hydrofluoric acids and loaded onto an anion exchange resin. Interferents were eluted using hydrochloric and hydrofluoric acids. The cadmium-containing fraction was eluted using nitric acid. This fraction was evaporated to dryness and redissolved in nitric acid prior to analysis. Lead was not homogeneously distributed in SRM 3233, with values ranging between 0.04 μg/g and 0.4 μg/g, and a value for lead could not be assigned (see "Homogeneity Assessment").

NIST Analysis for As Using RNAA: Arsenic was measured by RNAA using single 0.25 g test portions taken from each of six bottles of SRM 3233. Individual disks were formed from the test portions using a stainless steel die and hydraulic press. Samples were packaged individually in clean polyethylene bags and irradiated in one polyethylene irradiation vessel for 5 h at 20 MW, which provided a thermal neutron fluence rate of 3×10^{13} cm^{-2}s^{-1}. Samples were combined with ^{77}As prior to chemical separation. Samples were dissolved in a mixture of nitric and perchloric acids, and arsenic separated from the matrix as described in reference 7. The 559 keV line from decay of ^{76}As was used for quantitation. The 239 keV line from decay of ^{77}As was evaluated for yield determination.

NIST Analyses for Al, Cl, Cr, Fe, Mg, Mn, Mo, Na, V, and Zn Using INAA: Aluminum, chlorine, chromium, iron, magnesium, manganese, molybdenum, sodium, vanadium, and zinc were measured by INAA using duplicate 0.225 g test portions taken from each of six bottles of SRM 3233. Powders were pressed into cylindrical pellets, and samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually at 20 MW. For analysis of the short-lived nuclides (aluminum, chlorine, magnesium, manganese, sodium, and vanadium) by INAA, each sample, standard, or control material was individually irradiated together with one flux monitor foil for 60 s at a reactor power of 20 MW. The count was done after 5 min decay at a sample-to-detector distance of 14 cm for 5 min counting time. For the analysis of chromium, iron, molybdenum, and zinc samples by INAA, standards, and controls were irradiated for 4 h; irradiation capsules were then inverted 180 degrees, and materials were irradiated another 4 h. Molybdenum was counted for 8 h after a decay of more than 168 h. Chromium, cobalt, iron, and zinc were counted for 8 h after a decay of more than 120 days.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 3233 was based on the combination of results provided from various analytical methods at NIST, USDA, and collaborating laboratories.

NIST Analyses for Fat-Soluble Vitamins: Vitamin A (as retinyl palmitate) and vitamin E (as α-tocopheryl acetate) were measured at NIST using liquid chromatography with mass spectrometric detection (LC/MS). Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the fat-soluble vitamins in the SRM. Internal standards were employed; a single solution was used for the calibrants and samples.

Retinyl Palmitate and α-Tocopheryl Acetate: Duplicate 10 g test portions of powder from each of 12 bottles were accurately weighed into 50 mL polyethylene centrifuge tubes, and internal standard solutions containing tocol and retinyl palmitate-d_4 were added. Analytes were extracted into hexane by sonication and mixing/rotation for 60 min three times. Three additional extractions were performed using sonication in ethyl acetate and 60 min of mixing/rotation three times. The supernatants for the individual test portions were combined and were evaporated to approximately 25 mL under nitrogen. The extracts were washed with water, the organic phase was evaporated to

dryness, and the residue was reconstituted in ethanol. Separations were performed on a C_{18} column with an isocratic mobile phase of 60 % methanol and 40 % acetonitrile containing 5 mmol/L ammonium acetate. Retinyl palmitate, retinyl palmitate-d_4, tocol, and α-tocopheryl acetate were monitored at *m/z* 269, *m/z* 273, *m/z* 388, and *m/z* 473, respectively. Retinyl palmitate was not homogeneously distributed in SRM 3233, with values ranging between 2 μg/g and 12 μg/g, and a value for retinol could not be assigned.

NIST Analyses for Water-Soluble Vitamins: Water-soluble vitamins were measured by using LC methods with absorbance detection, MS, or isotope dilution (ID) tandem mass spectrometry (MS/MS). Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM. In cases where an internal standard was employed, a single solution was used for the calibrants and samples.

Ascorbic Acid: Ascorbic acid (vitamin C) was measured by LC using a C_{18} column with absorbance detection at 243 nm. Duplicate 2 g test portions from each of 10 bottles were dissolved in 30 g to 35 g of HPLC-grade water. An internal standard, 4-pyridoxic acid, was added. Metaphosphoric acid was added to stabilize the vitamin C in the mixture. Dithiothreitol was added to the mixture to convert dihydroascorbic acid to total ascorbic acid. The mixture was sonicated for 30 min and centrifuged at room temperature for 15 min. A 1 mL aliquot of the test mixture was removed and filtered using a 0.45 μm nylon filter prior to analysis using a gradient LC method with a potassium phosphate (dibasic)/acetonitrile mobile phase.

Thiamine, Riboflavin, Niacinamide, Niacin, Pantothenic Acid, Pyridoxine, and Pyridoxal: Thiamine, riboflavin, niacinamide, niacin, pantothenic acid, pyridoxine, and pyridoxal were measured by LC/MS in duplicate 0.5 g test portions taken from each of 12 bottles. Six internal standards were added: $^{13}C_3$-thiamine chloride; $^{2}H_4$-niacinamide; $^{2}H_4$-niacin; calcium $^{13}C_3$,^{15}N-pantothenate; $^{13}C_4$-pyridoxine hydrochloride; and $^{2}H_3$-pyridoxal hydrochloride. The analytes and internal standards were extracted into dilute acetic acid for analysis by positive-ion mode LC/MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C_{18} column were used for LC/MS determination of vitamins B_1 hydrochloride, B_2, and pyridoxine, niacinamide, niacin, pantothenic acid, and pyridoxal. Thiamine and $^{13}C_3$-thiamine were measured at *m/z* 265 and *m/z* 268, respectively. Niacinamide and $^{2}H_4$-niacinamide were measured at *m/z* 123 and *m/z* 127, respectively. Niacin and $^{2}H_4$-niacin were measured at *m/z* 124 and *m/z* 128, respectively. Pantothenic acid and $^{13}C_3$,^{15}N-pantothenic acid were measured at *m/z* 220 and *m/z* 224, respectively. Pyridoxine and $^{13}C_4$-pyridoxine were measured at *m/z* 170 and *m/z* 174, respectively. Pyridoxal and $^{2}H_3$-pyridoxal were measured at *m/z* 168 and *m/z* 171, respectively. Riboflavin was measured at *m/z* 377, with $^{13}C_4$-pyridoxine as the internal standard.

Cyanocobalamin: Cyanocobalamin (vitamin B_{12}) was measured in two 2.0 g test portions taken from each of six bottles. Cyanocobalamin was extracted into deionized water, samples were centrifuged, and the supernatants were filtered through 0.45 μm nylon filters. Yttrium was added as an internal standard. The samples were analyzed for cyanocobalamin, inorganic cobalt, and yttrium using a C_{18} column, a mobile phase of ethylenediaminetetraacetic acid in methanol and water, and inductively coupled plasma mass spectrometry (ICP-MS) with detection at *m/z* 59 for cyanocobalamin and *m/z* 89 for yttrium.

Folic Acid: Folic acid measurements were made on two 1.0 g test portions taken from each of 12 bottles. Internal standard $^{13}C_5$-folic acid was added. A sodium phosphate buffer containing ascorbic acid was added, and samples were vortex-mixed, subjected to gentle shaking at 37 °C, boiled, and cooled on ice. Supernatants from centrifuged samples were filtered through 0.45 μm filters. The filtered supernatants were analyzed for folic acid and $^{13}C_5$-folic acid by positive mode LC/MS/MS. A gradient LC method with a water/acetonitrile/formic acid mobile phase and a C_{18} reversed-phase column were used for the determination of both folic acid and $^{13}C_5$-folic acid. The transitions *m/z* 442.4 → *m/z* 295.1 and *m/z* 447.4 → *m/z* 295.1 were monitored for folic acid and $^{13}C_5$-folic acid, respectively.

USDA Analyses for Water-Soluble Vitamins: Thiamine, riboflavin, niacinamide, pantothenic acid, pyridoxine, and folic acid were measured by using ID-LC/MS. Thiamine, niacinamide, and pyridoxine were measured in the same sample extracts using hydrophilic interaction chromatography (HILIC) with ID-MS. Using newly prepared samples, the six vitamins were measured using ultra performance liquid chromatography (UPLC) methods with ID-MS. Results from the methods were similar and were therefore considered as a single data set, with the uncertainty as the standard error of the mean.

Collaborating Laboratories' Analyses: The GMA FIACC laboratories were asked to use their usual methods to make single measurements of proximates, calories, vitamins, elements, and amino acids on test portions taken from each of two bottles of SRM 3233. In a second exercise, a subset of these laboratories measured sugars in each of two bottles. In a third exercise, several GMA laboratories and one other laboratory measured dietary fiber in each of six bottles using four AOAC Official Methods of Analysis: 985.29 Total Dietary Fiber in Foods (Enzymatic-Gravimetric Method); 991.42 Insoluble Dietary Fiber in Foods and Food Products (Enzymatic-Gravimetric Method, Phosphate Buffer); 2009.01 Total Dietary Fiber in Foods

(Enzymatic-Gravimetric-Liquid Chromatographic Method); and 2011.25 Insoluble, Soluble, and Total Dietary Fiber in Foods (Enzymatic-Gravimetric-Liquid Chromatography) [8]. Because of variability among data provided by laboratories participating in an interlaboratory comparison exercise, the median of laboratory means is used, with the uncertainty estimated using the median absolute deviation (MADe) [9].

Determination of Moisture: Moisture content of SRM 3233 was determined at NIST (see "Instructions for Storage and Use") by (1) freeze-drying to constant mass over 7 days; (2) drying over magnesium perchlorate in a desiccator at room temperature for 28 days; and (3) drying for 2 h in a forced-air oven at 80 °C. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of (0.983 ± 0.007) gram dry mass per gram as-received mass, which was used to convert data from an as-received to a dry-mass basis; the uncertainty shown on this value is an expanded uncertainty. An uncertainty component for the conversion factor (0.36 %) obtained from the moisture measurements is incorporated in the uncertainties of the certified and reference values, reported on a dry-mass basis, that are provided in this certificate.

Homogeneity Assessment: The homogeneity of vitamins and elements was assessed at NIST using the methods and test portion sizes described above. Homogeneity of constituents measured solely by collaborating laboratories (e.g., proximates) was not assessed, although the data were treated as through these analytes were homogeneously distributed. Certain elements showed a lack of homogeneity, possibly due to wear metal contamination during grinding; in these cases, either values are not provided (chromium, lead, nickel, tin) or the uncertainties incorporate a component for inhomogeneity (cobalt, molybdenum). For the other elements and the vitamins, analysis of the variance did not show statistically significant heterogeneity.

Value Assignment: The collaborating laboratories reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. 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 also measured by NIST, the median of the individual collaborating laboratory means, the USDA's mean, and the mean of the individual sets of NIST data were averaged, as appropriate.

Certified Mass Fraction Values for Selected Elements: Each certified mass fraction value is the mean from the combination of the mean of results from analyses by NIST and the median of the mean of results provided by collaborating laboratories, where available. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties and an uncertainty component for moisture correction, consistent with the ISO Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4].

Table 1. Certified Mass Fraction Values (Dry-Mass Basis) for Selected Elements in SRM 3233

	Mass Fraction (mg/kg)			Coverage Factor, k
Barium[a,b]	2.766	±	0.033	2.00
Cadmium[c]	0.0819	±	0.0020	2.15
Calcium[a,d]	36910	±	920	2.00
Copper[a,d]	3.97	±	0.28	2.00
Iron[a,d,e]	766	±	36	2.00
Magnesium[a,d,e]	1093	±	37	2.00
Manganese[a,d,e]	33.1	±	1.1	2.00
Phosphorus[a,d]	2592	±	68	2.00
Potassium[a,d]	3060	±	140	2.00
Sodium[a,d,e]	6830	±	120	2.00
Strontium[a,b]	8.34	±	0.17	2.00
Zinc[a,d,e]	628	±	16	2.00

[a] NIST ICP-OES
[b] NIST ICP-MS
[c] NIST ID ICP-MS
[d] Collaborating laboratories
[e] NIST INAA

Certified Mass Fraction Values for Selected Vitamins: Each certified mass fraction value is the mean from the combination of the mean results from each set of analyses by NIST, the median of the mean of results provided by collaborating laboratories, and the mean result provided by the material manufacturer, where available. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties and an uncertainty component for moisture correction, consistent with the ISO Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4].

Table 2. Certified Mass Fraction Values (Dry-Mass Basis) for Selected Vitamins in SRM 3233

	Mass Fraction (mg/kg)	Coverage Factor, k
Thiamine (Vitamin B_1)[a,b,c,e]	60.2 ± 9.4	2.00
Riboflavin (Vitamin B_2)[b,d,e]	76 ± 2	2.00
Niacinamide[c,e]	799 ± 27	2.00
Total Vitamin B_3 as Niacinamide[b,c,g]	822 ± 39	2.00
Pantothenic Acid[b,c,e]	540 ± 40	2.00
Pyridoxine[c,e]	78.0 ± 4.7	2.00
Total Vitamin B_6 as Pydridoxine[b,c,h]	81.9 ± 9.0	2.00
Folic Acid[b,e,f]	15.1 ± 1.2	2.00
Total α-Tocopherol (Vitamin E)[b,d,i]	1350 ± 220	2.00

[a] Reported as thiamine ion (265.36 g/mol), not thiamine chloride or thiamine chloride hydrochloride.
[b] Collaborating laboratories
[c] NIST ID-LC/MS
[d] NIST LC/MS
[e] USDA
[f] NIST ID-LC/MS/MS
[g] Measured as the sum of niacinamide and niacin, which was mathematically converted to niacinamide by multiplication by the ratio of the relative molecular masses.
[h] Measured as the sum of pyridoxine and pyridoxal, which was mathematically converted to pyridoxine by multiplication by the ratio of the relative molecular masses.
[i] α-Tocopherol was added to SRM 3233 as RRR-α-tocopheryl acetate. This certified value is expressed as α-tocopherol equivalents and includes "naturally occurring" α-tocopherol as well as the α-tocopherol acetate that was added.

Reference Mass Fraction Values for Selected Elements: Each reference mass fraction value is the mean result of NIST analyses using one or two methods. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty, incorporating an uncertainty component for moisture correction, consistent with the ISO Guide, and k is a coverage factor corresponding to approximately 95 % confidence [2]. The uncertainties for cobalt and molybdenum also incorporate an additional uncertainty component for possible inhomogeneity.

Table 3. Reference Mass Fraction Values (Dry-Mass Basis) for Selected Elements in SRM 3233

	Mass Fraction (mg/kg)	Coverage Factor, k
Cobalt[a]	0.174 ± 0.033	2.57
Molybdenum[a]	1.61 ± 0.16	2.57
Vanadium[a,b]	0.297 ± 0.040	2.00

[a] NIST ICP-MS
[b] NIST INAA

Reference Mass Fraction Values for Selected Vitamins: Each reference mass fraction value is the mean result of a NIST analysis using a single method or the mean from the combination of NIST results with the median of the mean results provided by collaborating laboratories, where available. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty, incorporating an uncertainty component for

moisture correction, consistent with the ISO Guide, and k is a coverage factor corresponding to approximately 95 % confidence [2]. For values based on more than one data source, the combined uncertainty incorporates the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO Guide and with its Supplement 1 [2–4].

Table 4. Reference Mass Fraction Values (Dry-Mass Basis) for Selected Vitamins in SRM 3233

	Mass Fraction (mg/kg)	Coverage Factor, k
Ascorbic Acid (Vitamin C)[a,b]	2440 ± 620	2.00
Niacin[c]	16.67 ± 0.35	2.14
Pyridoxal[c]	2.25 ± 0.19	2.20
Cyanocobalamin (Vitamin B_{12})[b,d]	0.210 ± 0.040	2.00

[a] NIST LC/absorbance
[b] Collaborating laboratories
[c] NIST ID-LC/MS
[d] NIST LC-ICP-MS

Reference Values for Proximates, Sugars, Dietary Fiber, and Calories: Each reference value is the median of the mean results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty, incorporating an uncertainty component for moisture correction, consistent with the ISO Guide, and k is a coverage factor corresponding to approximately 95 % confidence [2].

Table 5. Reference Values (Dry-Mass Basis) for Proximates, Sugars, and Calories in SRM 3233

	Mass Fraction (%)	Coverage Factor, k
Solids	99.09 ± 0.79	2.00
Ash	11.87 ± 0.25	2.11
Protein[a]	7.25 ± 0.18	2.13
Fat (as the sum of fatty acids as triglycerides)	2.02 ± 0.40	2.16
Hexadecanoic Acid (C16:0) (Palmitic Acid)	0.367 ± 0.072	2.23
Octadecanoic Acid (C18:0) (Stearic Acid)	0.173 ± 0.051	2.23
(Z)-9-Octadecenoic Acid (C18:1 n-9) (Oleic Acid)	0.278 ± 0.027	2.26
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) (Linoleic Acid)	0.867 ± 0.155	2.26
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) (α-Linolenic Acid)	0.056 ± 0.007	2.31
Carbohydrates	77.88 ± 0.86	2.03
Total Sugars	15.8 ± 1.5	2.78
Fructose	0.81 ± 0.39	2.78
Glucose	1.04 ± 0.36	2.78
Maltose	0.46 ± 0.09	4.30
Sucrose	13.42 ± 0.75	2.78
	Energy (kcal per 100 g)	**Coverage Factor, k**
Calories[b]	362.4 ± 3.8	2.03

[a] A factor of 5.7 was used to convert nitrogen results to protein.
[b] The reference value for calories is the median of lab mean caloric calculations from the interlaboratory comparison exercise. If the mean proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 358.7 kcal/100 g.

Table 6. Reference Mass Fraction Values (Dry-Mass Basis) for Dietary Fiber in SRM 3233[a]

	Mass Fraction (%)	Coverage Factor, k
Composite Data for Dietary Fiber Obtained Using Four AOAC Methods[b]		
IDF + HMW SDF	9.19 ± 0.94	2.78
IDF	6.60 ± 0.45	2.78
LMW SDF	3.02 ± 0.61	2.78
HMW SDF	2.87 ± 0.61	2.78
TDF	12.24 ± 0.78	2.78
Based on Data Obtained Using AOAC 2011.25[c]		
IDF	6.6 ± 1.3	4.30
LMW SDF	3.0 ± 1.2	4.30
HMW DF	2.6 ± 1.5	4.30
TDF	11.9 ± 2.7	4.30
Based on Data Obtained Using AOAC 2009.01		
IDF + HMW SDF[b]	9.19 ± 0.94	2.78
LMW SDF[d]	2.92 ± 0.61	2.00
TDF[d]	12.53 ± 0.58	2.00
Based on Data Obtained Using AOAC 991.43		
SDF[d]	2.71 ± 0.84	2.00
TDF[d]	9.0 ± 1.2	2.00

[a] DF = dietary fiber
IDF = insoluble dietary fiber
HMW = high molecular weight
LMW = low molecular weight
SDF = soluble dietary fiber
TDF = total dietary fiber

[b] Data reported by five laboratories.
[c] Data reported by three laboratories.
[d] Data reported by two laboratories.

Reference Mass Fraction Values for Amino Acids: Each reference mass fraction value is the median of the mean results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty, incorporating an uncertainty component for moisture correction, consistent with the ISO Guide, and k is a coverage factor corresponding to approximately 95 % confidence [2].

Table 7. Reference Mass Fraction Values (Dry-Mass Basis) for Amino Acids in SRM 3233

	Mass Fraction (%)			Coverage Factor, k
Alanine	0.323	±	0.042	2.57
Arginine	0.322	±	0.067	2.57
Aspartic Acid	0.438	±	0.050	2.57
Cysteine	0.154	±	0.032	3.18
Glutamic Acid	2.25	±	0.22	2.57
Glycine	0.342	±	0.031	2.57
Histidine	0.162	±	0.034	2.57
Isoleucine	0.270	±	0.014	2.57
Leucine	0.550	±	0.047	2.57
Lysine	0.103	±	0.040	2.57
Methionine	0.139	±	0.019	2.78
Phenylalanine	0.373	±	0.033	2.57
Serine	0.375	±	0.062	2.57
Threonine	0.241	±	0.013	2.57
Tryptophan	0.092	±	0.045	3.18
Tyrosine	0.231	±	0.058	2.57
Valine	0.343	±	0.026	2.57

Information Mass Fraction Values for Selected Elements: Each information mass fraction value is the mean result of a NIST analysis using a single method. No uncertainty is provided because there is insufficient information available for its assessment.

Table 8. Information Mass Fraction Values (Dry-Mass Basis) for Selected Elements in SRM 3233

	Mass Fraction (mg/kg)
Aluminum[(a)]	40
Chlorine[(a)]	10 000
Iodine[(b)]	0.04

	Mass Fraction (µg/kg)
Arsenic[(c)]	80

[(a)] NIST INAA
[(b)] NIST ICP-MS
[(c)] NIST RNAA

Table 9. Information Mass Fraction Values (Dry-Mass Basis) for Dietary Fiber in SRM 3233[a,b]

	Mass Fraction (%)
Based on Data Obtained Using AOAC 2009.01	
HMW DF	9.59
Based on Data Obtained Using AOAC 991.43	
IDF	6.41
Based on Data Obtained Using AOAC 985.29	
IDF	6.61
HMW SDF	2.98

[a] DF = dietary fiber
IDF = insoluble dietary fiber
HMW = high molecular weight
SDF = soluble dietary fiber

[b] Data reported by one laboratory; insufficient information is available to assign an uncertainty to this value.

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Certificate Revision History: 12 February 2013 (Changed unit size; removed test portion size for fiber analysis; editorial changes); 28 September 2012 (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.