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: Certified mass fraction values for elements and vitamins in SRM 3233, reported on a dry-mass basis, are provided in Tables 1 and 2. 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 values 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].

Reference Mass Fraction Values: Reference mass fraction values for additional elements and vitamins, proximates, sugars, calories, dietary fiber, and amino acids in SRM 3233, reported on a dry-mass basis, are provided in Tables 3 through 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 an uncertainty 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 and/or collaborating laboratories.

Information Mass Fraction Values: Information mass fraction values for additional elements and dietary fiber, reported on a dry-mass basis, are provided in Tables 8 and 9 to help characterize the composition of the material. 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 and therefore no uncertainty is provided. Values are reported on a dry-mass basis in mass fraction units [5]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 3233** is valid, within the measurement uncertainty specified, until **20 June 2027**, 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 or register online) will facilitate notification.

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

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Steven J. Choquette, 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 (FIACC) 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); Megazyme International Ireland Ltd (Bray, County Wicklow, Ireland); Schwan Food Company (Salina, KS); Silliker (Madison, WI); The J.M. Smucker Co. (Orrville, OH); The National Food Laboratory (Livermore, CA).

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 AND WARNING TO USERS

SRM 3233 IS INTENDED FOR RESEARCH USE, 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 recapped and test portions removed and analyzed until the material reaches its expiration date. 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). For analyses of remaining analytes, the bottle can be recapped and test portions removed and analyzed for three months after the bottle was first opened.

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 portion size should be based on descriptions below (See "Source, Preparation, and 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 should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 6.

Determination of Moisture: The moisture content of SRM 3233 was determined at NIST by (1) freeze-drying to constant mass over 7 d; (2) drying over magnesium perchlorate in a desiccator at room temperature for 28 d; 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 ($k = 2$) to represent a 95 % level of confidence. 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.

SOURCE, PREPARATION, AND ANALYSIS[1]

Source and Preparation: The SRM is a fortified breakfast cereal. Two hundred kilograms (440 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 aluminized 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.

[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.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of elements in SRM 3233 was based on the combination of measurements from two different analytical methods at NIST and data from 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).

NIST Analyses for Ba, Ca, Co, Cu, Fe, I, K, Mg, Mn, Mo, Na, P, Sr, V, and Zn Using ICP-OES and/or ICP-MS: Mass fractions of 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. Mass fractions of barium, cobalt, molybdenum, strontium, 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. The mass fraction of 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. Quantification for all elements measured by ICP-OES and ICP-MS was based on the method of standard additions using SRM 3100 series single element standard solutions.

NIST Analysis for Cd Using ID ICP-MS: The mass fraction of cadmium was 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 were digested in nitric acid using a microwave sample preparation system. To remove spectral interferences in the cadmium determination, 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. Quantification for ID ICP-MS was based on calibration by reverse isotope dilution ICP-MS using primary standards prepared from high-purity Cd metals as well as *SRM 3108 Cadmium (Cd) Standard Solution*.

NIST Analysis for As Using RNAA: The mass fraction of 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, then 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 quantification. The 239 keV line from decay of ^{77}As was evaluated for yield determination. Quantification for RNAA was based on solutions made using *SRM 3103a Arsenic (As) Standard Solution*.

NIST Analyses for Al, Cl, Fe, Mg, Mn, Mo, Na, V, and Zn Using INAA: Mass fractions of aluminum, chlorine, 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, the packages were irradiated with one flux monitor foil for 60 s at a reactor power of 20 MW. The count was done after a 5 min decay at a sample-to-detector distance of 14 cm for a 5 min counting time. For the analysis of iron, molybdenum, and zinc by INAA, samples, 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. Cobalt, iron, and zinc were counted for 8 h after a decay of more than 120 days. Quantification for INAA was based on solutions made using SRM 3100 series single element standard solutions.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of vitamins in SRM 3233 was based on the combination of measurements from various analytical methods at NIST, USDA, and collaborating laboratories, where available. NIST provided measurements by using liquid chromatography (LC) with mass spectrometry (MS) and absorbance detection, as well as by using isotope dilution (ID) LC with MS or tandem mass spectrometry (MS/MS) detection.

NIST Analysis for α-Tocopheryl Acetate Using LC-MS: The mass fraction of α-tocopheryl acetate was measured in duplicate 10 g test portions taken from each of 12 bottles of SRM 3233. Ethanolic tocol was added as an internal standard. The analyte and internal standard were extracted from the sample into hexane by rotational agitation for 60 min. The samples were centrifuged, the supernatants were decanted, and an additional aliquot of hexane was added. Samples were extracted further by 60 min of rotational agitation. An additional cycle of rotational agitation

was conducted, for a total of three extractions. Three additional extractions were performed using sonication in ethyl acetate followed by 60 min of rotational agitation. The pooled organic layers were evaporated to approximately 25 mL under nitrogen, washed with water, evaporated to dryness under nitrogen, and resuspended in ethanol for analysis by LC-MS. Separations were performed on a C18 column with an isocratic mobile phase of 60 % methanol and 40 % acetonitrile (volume fractions) containing 5 mmol/L ammonium acetate. Tocol and α-tocopheryl acetate were monitored at *m/z* 388 and *m/z* 473, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the level of α-tocopheryl acetate in the SRM following extraction. The purity of the α-tocopheryl acetate calibrant material was determined at NIST using spectrophotometry at 284 nm (molar extinction coefficient of 43.6 $dL \cdot g^{-1}cm^{-1}$) in combination with LC-absorbance at 284 nm. A single internal standard solution was used for the calibrants and samples.

NIST Analysis for Ascorbic Acid Using LC-Absorbance: The mass fraction of ascorbic acid (vitamin C) was measured in duplicate 2 g test portions taken from each of 10 bottles of SRM 3233. 4-pyridoxic acid was added as an internal standard, metaphosphoric acid was added to stabilize the vitamin C in solution, and dithiothreitol was added to convert dihydroascorbic acid to total ascorbic acid. The analyte and internal standard were extracted from the sample into 30 g to 35 g of HPLC-grade water by sonication for 30 min. Following centrifugation, a 1 mL test portion was removed and filtered using a 0.45 µm nylon filter for analysis by LC-absorbance. Separations were performed on a C18 column with a gradient mobile phase of potassium phosphate (dibasic) and acetonitrile and absorbance detection at 243 nm. The purity of the neat calibrant material was determined at NIST using LC-absorbance at 243 nm. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Thiamine, Niacinamide, Niacin, and Pantothenic Acid Using ID-LC-MS: The mass fractions of thiamine, niacinamide, niacin, and pantothenic acid were measured in duplicate 0.5 g test portions taken from each of 12 bottles of SRM 3233. Four internal standards were added: $^{13}C_3$-thiamine chloride; $^{2}H_4$-niacinamide; $^{2}H_4$-niacin; and calcium $^{13}C_3$,^{15}N-pantothenate. The analytes and internal standards were extracted into dilute acetic acid by sonication for 30 min. Following centrifugation, a 1 mL test portion was removed and filtered using a 0.45 µm regenerated cellulose filter for analysis by positive-ion mode ID-LC-MS. Separations were performed on a C18 column with a gradient mobile phase of ammonium formate buffer and methanol. 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. The purity of neat calibrant materials was determined at NIST using LC-absorbance, Karl Fischer titration, thermogravimetric analysis, and differential scanning calorimetry. A single internal standard solution was used for the calibrants and samples.

NIST Analysis for Folic Acid Using ID-LC-MS/MS: The mass fraction of folic acid was measured in duplicate 1.0 g test portions taken from each of 12 bottles of SRM 3233. $^{13}C_5$-folic acid was added as an internal standard. The analyte and internal standard were extracted into a sodium phosphate buffer containing ascorbic acid by vortex mixing, gentle shaking at 37 °C for 2 h, and boiling for 15 min. Extracts were then cooled on ice and a test portion filtered through a 0.45 µm polyvinylidene difluoride (PVDF) filter for analysis by positive-ion mode ID-LC-MS/MS. Separations were performed on a C18 column with a gradient mobile phase of water and acetonitrile containing formic acid. Folic acid and $^{13}C_5$-folic acid were monitored at the transitions *m/z* 442.4 → *m/z* 295.1 and *m/z* 447.4 → *m/z* 295.1, respectively.

USDA Analyses for Water-Soluble Vitamins: Mass fractions of thiamine, niacinamide, pantothenic acid, and folic acid were measured by using ID-LC-MS. Mass fractions of thiamine and niacinamide were measured using hydrophilic interaction chromatography (HILIC) with ID-MS. Mass fractions of thiamine, niacinamide, pantothenic acid, and folic acid were measured in separate samples 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].

Homogeneity Assessment: The homogeneity of vitamins and elements was assessed at NIST using the methods and test portion sizes described above. Analysis of the variance showed statistically significant heterogeneity for cobalt and molybdenum, and the uncertainties for these values incorporate an additional component for possible heterogeneity. Homogeneity of constituents measured solely by collaborating laboratories (e.g., proximates, amino acids) was not assessed, although the data were treated as though these analytes were homogeneously distributed.

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. 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 mean of the individual sets of NIST data were averaged with the mean of USDA means and the median of the individual GMA FIACC laboratory means, as appropriate.

Certified Mass Fraction Values for Elements: Each certified mass fraction value is the combined mean from the means of results from analyses by NIST and the median of the means 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/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurand is the total mass fraction for each element listed in Table 1 on a dry-mass basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

Table 1. Certified Mass Fraction Values for Elements in SRM 3233

	Mass Fraction (mg/kg)	Coverage Factor, k
Barium (Ba)[a,b]	2.766 ± 0.033	2.00
Cadmium (Cd)[c]	0.0819 ± 0.0020	2.15
Calcium (Ca)[a,d]	36910 ± 920	2.00
Copper (Cu)[a,d]	3.97 ± 0.28	2.00
Iron (Fe)[a,d,e]	766 ± 36	2.00
Magnesium (Mg)[a,d,e]	1093 ± 37	2.00
Manganese (Mn)[a,d,e]	33.1 ± 1.1	2.00
Phosphorus (P)[a,d]	2592 ± 68	2.00
Potassium (K)[a,d]	3060 ± 140	2.00
Sodium (Na)[a,d,e]	6830 ± 120	2.00
Strontium (Sr)[a,b]	8.34 ± 0.17	2.00
Zinc (Zn)[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 Vitamins: Each certified mass fraction value is the combined mean from the means of results from NIST analyses, the median of the means of results provided by collaborating laboratories, and the mean results provided by the USDA, 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/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurand is the total mass fraction for each vitamin listed in Table 2 on a dry-mass basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

Table 2. Certified Mass Fraction Values for Vitamins in SRM 3233

	Mass Fraction (mg/kg)	Coverage Factor, k
Thiamine (Vitamin B_1)[a,b,c,d]	60.2 ± 9.4	2.00
Niacinamide (Vitamin B_3)[c,d]	799 ± 27	2.00
Total Vitamin B_3 as Niacinamide[c,e,f]	822 ± 39	2.00
Pantothenic Acid (Vitamin B_5)[c,d,e]	540 ± 40	2.00
Folic Acid[d,e,g]	15.1 ± 1.2	2.00
Total α-Tocopherol (Vitamin E)[h,i,j]	1350 ± 220	2.00

[a] Reported as thiamine ion (265.36 g/mol), not thiamine chloride or thiamine chloride hydrochloride.
[b] Collaborating laboratories. Reported methods include digestion with fluorescence detection and extraction with LC and fluorescence detection.
[c] NIST ID-LC-MS
[d] USDA
[e] Collaborating laboratories. Reported methods include microbiological assay and enzymatic digestion with LC-MS/MS.
[f] Measured as the sum of niacinamide and niacin, which was mathematically converted to niacinamide by multiplication by the ratio of the relative molecular masses.
[g] NIST ID-LC-MS/MS
[h] NIST LC-MS
[i] Collaborating laboratories.
[j] Vitamin E 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 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 consistent with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2-4]. The uncertainties for cobalt and molybdenum also incorporate an additional uncertainty component for possible inhomogeneity. The measurand is the mass fraction for each element listed in Table 3, on a dry-mass basis, as determined by the method indicated. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram), as realized by the methods used.

Table 3. Reference Mass Fraction Values for Elements in SRM 3233

	Mass Fraction (mg/kg)	Coverage Factor, *k*
Cobalt (Co)[a]	0.174 ± 0.033	2.57
Molybdenum (Mo)[a]	1.61 ± 0.16	2.57
Vanadium (V)[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 NIST analyses 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 consistent with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2-4]. The measurand is the mass fraction for each vitamin listed in Table 4, on a dry-mass basis, as determined by the method indicated. Metrological traceability is to mass fraction (expressed as milligrams per kilogram), as realized by the methods used.

Table 4. Reference Mass Fraction Values for 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

[a] NIST LC-absorbance
[b] Collaborating laboratories. Reported methods include colorimetric titration and LC with absorbance or fluorescence detection.
[c] NIST ID-LC-MS

Reference Values for Proximates, Sugars, and Calories: Each reference value is the median of the means of 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 consistent with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. For proximates and sugars, the measurands are the mass fractions listed in Table 5, on a dry-mass basis, as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams), as realized by the methods used. For calories, the measurand is the caloric content (expressed as kilocalories per 100 grams), listed in Table 5, on a dry-mass basis as determined by the method indicated, and metrological traceability is to the scale realized by that method for energy.

Table 5. Reference Mass Fraction Values for Proximates, Sugars, and Calories in SRM 3233

	Mass Fraction (g/100 g)	Coverage Factor, k
Ash[a]	11.87 ± 0.25	2.11
Protein[b]	7.25 ± 0.18	2.13
Fat (as the sum of fatty acids as triglycerides)	2.02 ± 0.40	2.16
Carbohydrates[c]	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[d]	362.4 ± 3.8	2.03

[a] Ash was determined by collaborating laboratories using weight loss after ignition in a muffle furnace.
[b] Nitrogen was determined by collaborating laboratories using Kjeldahl, thermal conductivity, pyrolysis with gas chromatography, pyrolysis with thermal conductivity, and combustion. A factor of 5.7 was used to convert nitrogen results to protein.
[c] Carbohydrates were determined by collaborating laboratories by difference (solids less the sum of protein, fat, and ash).
[d] Calories were determined by collaborating laboratories as the sum of caloric equivalents of 9, 4, and 4 for fat (as the sum of fatty acids as triglycerides), protein, and carbohydrate, respectively. If the mean proximate values above are used for calculation, the mean caloric content is 358.7 kcal per 100 grams.

Reference Values for Dietary Fiber: Each reference value is the median of the means of 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 consistent with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. For dietary fiber, the measurands are the mass fractions of dietary fiber, listed in Table 6, on a dry-mass basis as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams), as realized by the methods used.

Table 6. Reference Mass Fraction Values for Dietary Fiber in SRM 3233[a]

	Mass Fraction (g/100 g)	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[d]		
SDF	2.71 ± 0.84	2.00
TDF	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 using hydrolysis followed by derivatization and LC. 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 consistent with the ISO/JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. The measurand is the mass fraction of each amino acid, listed in Table 7, on a dry-mass basis as determined by the method indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams), as realized by the methods used.

Table 7. Reference Mass Fraction Values for Amino Acids in SRM 3233

	Mass Fraction (g/100 g)	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 Elements: Each information mass fraction value, reported on a dry-mass basis, 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. Information values cannot be used to establish metrological traceability.

Table 8. Information Mass Fraction Values for 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

Information Mass Fraction Values for Dietary Fiber: Each information mass fraction value, reported on a dry-mass basis, is the median of the mean results provided by a single collaborating laboratory using a single method. No uncertainty is provided because there is insufficient information available for its assessment. Information values cannot be used to establish metrological traceability.

Table 9. Information Mass Fraction Values for Dietary Fiber in SRM 3233[(a)]

	Mass Fraction (g/100 g)
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

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Certificate Revision History: 14 June 2017 (Change of expiration date; removal of values for riboflavin (vitamin B_2), pyridoxine, pyridoxal, cyanocobalamin (vitamin B_{12}), and fatty acids; editorial changes); **05 September 2014** (Removed reference value for solids; editorial changes); **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.