National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1849a

Infant/Adult Nutritional Formula I
(milk-based)

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining proximates, fatty acids, cholesterol, vitamins, elements, amino acids, and nucleotides in infant and adult nutritional formulas and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a milk-based, hybrid infant/adult nutritional powder prepared by a manufacturer of infant formula and adult nutritional products. A unit of SRM 1849a consists of 10 packets, each containing approximately 10 g of material.

Certified Mass Fraction Values: Certified mass fraction values for fatty acids, cholesterol, elements, vitamins, and carnitine in SRM 1849a, reported on an as-received basis, are provided in Tables 1 through 3. 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 taken into account [1]. Certified values were calculated as the unweighted mean of the mean values from NIST methods, the median of the mean results provided by collaborating laboratories, and the mean provided by the manufacturer, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2–4].

Reference Mass Fraction Values: Reference mass fraction values for additional fatty acids, proximates, lactose monohydrate, calories, additional vitamins, *myo*-inositol, amino acids, taurine, and nucleotides in SRM 1849a, reported on an as-received basis, are provided in Tables 4 through 8. 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 associated 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, collaborating laboratories, or the material manufacturer.

Information Mass Fraction Values: Information values for free choline ion and nucleotides plus nucleosides 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 [1]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 1849a** is valid, within the measurement uncertainty specified, until **30 November 2021**, 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, K.E. Sharpless, and L.J. Wood of the NIST Chemical Sciences Division, S. Ehling of the Grocery Manufacturers Association (GMA, Washington, DC), and M.K. Mountford of the Infant Nutrition Council of America (Atlanta, GA).

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Certificate Issue Date: 13 July 2018
Certificate Revision History on Last Page

Analytical measurements at NIST were performed by T.A. Butler, J. Camara, B.E. Lang, R. Oflaz, M.M. Phillips, B.J. Place, S.A. Rabb, C.A. Rimmer, J.R. Sieber, L.T. Sniegoski, J.B. Thomas, M.J. Welch, and L.J. Wood of the NIST Chemical Sciences Division.

Collaborating Laboratories: Analysts at the following laboratories analyzed SRM 1849a for value assignment as part of a GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Abbott Nutrition (Columbus, OH); Campbell Soup Company (Camden, NJ); Conagra Foods (Omaha, NE); Covance, Inc. (Madison, WI); Del Monte Foods (Walnut Creek, CA); Eurofins Chemical Control (Cuneo, Italy); Eurofins Central Analytical Laboratories (Metairie, LA); Eurofins Scientific (Des Moines, IA); General Mills, Inc. (Golden Valley, MN); Hormel Foods Corporation (Austin, MN); Land O'Lakes (Arden Hills, MN); Nestlé USA (Dublin, OH); Schwan Food Company (Salina, KS); Silliker (Madison, WI); Silliker Shanghai Ltd. (Shanghai, China); Silliker Canada (Markham, ON, Canada); Silliker Ibérica (Barcelona, Spain); and The J.M. Smucker Co. (Orrville, OH). As part of a separate interlaboratory comparison exercise organized through the International Formula Council, the following laboratories also provided results that were combined with data from the GMA FIACC laboratories: Fonterra (Waitoa, New Zealand), and Nestlé (Nunspeet, The Netherlands). Analyses for value assignment were also performed by Hong Kong Government Laboratory.

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 1849a IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION. THIS MATERIAL CONTAINS SOME NUTRIENTS AT LEVELS NOT PERMITTED IN INFANT FORMULA AND IS NOT AN INFANT FORMULA.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The original unopened packets of SRM 1849a should be stored at –80 °C or lower. For organic constituents, the certification only applies to the initial use, and the same results are not guaranteed if the remaining powder is used at a later date. For inorganic constituents, an open packet can be reused until the material reaches its expiration date, provided the at the open packet is resealed and stored at –80 °C or lower.

Use: Before use, shake the unopened packet to ensure the contents are mixed thoroughly. For certified values to be valid, the test portion mass indicated in the description of the NIST analyses for each group of analytes below should be used (see "Source, Preparation, and Analysis"). The stability of organic analytes in previously opened and stored packets has not been investigated. 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 milk-based, hybrid infant/adult nutritional powder, prepared by a manufacturer of infant formula and adult nutritional products. A base liquid containing all constituents was conventionally heat processed, homogenized, and spray-dried. The product was packaged into single-use nitrogen-flushed pouches, each containing 10 g of powder. The material was stored below 0 °C following packaging and is stored at NIST at –80 °C to enhance long-term stability.

Analytical Approach for Determination of Fatty Acids and Cholesterol: Value assignment of the mass fractions of fatty acids in SRM 1849a was based on the combination of measurements made at NIST using gas chromatography (GC) with flame ionization detection (FID) and by collaborating laboratories. Value assignment of the cholesterol mass fraction was based on measurements made by NIST using an isotope dilution (ID) GC method with mass spectrometric (MS) detection.

NIST Analyses for Fatty Acids Using GC-FID: Mass fractions of fatty acids were measured by GC-FID from single 0.5 g test portions from each of 7 packets of SRM 1849a. Samples were combined with wet Hydromatrix (Varian, Palo Alto, CA) and transferred to a glass extraction thimble. An internal standard solution containing tridecanoic acid

[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.

triglyceride and octacosanoic acid methyl ester was added, and samples were extracted for 22 h using a hexane/acetone (4:1, volume fraction) solution. Following extraction, extracts were concentrated in toluene, and 1 mL of MethPrep II (0.1 mol/L methanolic [*m*-trifluoromethylphenyl] trimethylammonium hydroxide, Alltech, Deerfield, IL) was added. Samples were mixed for 10 s to 15 s and allowed to equilibrate for at least 1 h prior to analysis by GC-FID. Six independently prepared calibrants were used for quantitation. GC-FID was performed using a 0.25 mm × 100 m biscyanopropyl polysiloxane fused silica capillary column. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in Isooctane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on linear regression of response factors for the calibrants.

NIST Analyses for Cholesterol Using ID-GC-MS: The mass fraction of cholesterol was measured using the ID-GC-MS method developed at NIST for serum cholesterol [7] and modified for the determination of cholesterol in food matrices using AOAC International Official Method 996.06 for hydrolysis [8]. Three sets of samples were prepared. Each set consisted of duplicate 0.5 g test portions from each of five packets of SRM 1849a weighed into round-bottom flasks. An aliquot of a solution containing a known mass of the internal standard, cholesterol-$^{13}C_3$, was added to each flask. Hydrolysis of cholesterol esters was accomplished by refluxing the samples in an alcohol-KOH solution for 1 h. Hexane was then used to extract the cholesterol. A portion of the hexane extract was evaporated to dryness and N,O bis-(trimethylsilyl)acetamide was added to convert cholesterol to its trimethylsilyl (TMS) derivative (cholesterol-TMS). Analyses were performed on a GC-MS system operated in the electron ionization mode with selected ion monitoring at *m/z* 458 and *m/z* 461 for the unlabeled and labeled cholesterol-TMS, respectively. The GC was equipped with a 30 m (5:95 phenyl/methyl polysiloxane [mole fraction]) non-polar fused silica column directly interfaced to the ion source. Standards consisting of mixtures of known quantities of pure unlabeled cholesterol (SRM 911c *Cholesterol*) and cholesterol-$^{13}C_3$ were run before and after the samples to generate composite linear regressions for calculation of the quantity of cholesterol in the samples.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of vitamins in SRM 1849a were based on the combination of results provided from various analytical methods at NIST, collaborating laboratories, and the manufacturer, where available. NIST provided measurements by using ID with liquid chromatography (LC) and MS or tandem MS (MS/MS), LC with absorbance detection, and LC with fluorescence detection.

NIST Analyses for Retinyl Palmitate, Cholecalciferol, and Phylloquinone Using ID-LC-MS: Mass fractions of vitamin A (as retinyl palmitate), vitamin D_3 (cholecalciferol), and vitamin K_1 (phylloquinone) were measured by ID-LC-MS from duplicate 3 g to 5 g test portions of SRM 1849a from each of 10 packets. Samples were accurately weighed into 50 mL polyethylene centrifuge tubes and aliquots of internal standard solutions containing retinyl palmitate-d_4, vitamin D_3-d_3, and vitamin K_1-d_6 were added. Analytes were extracted into ethyl acetate by sonication and then mixing/rotation overnight. Five additional extractions were performed using sonication and 30 min of mixing/rotation. The supernatants for the individual test portions were combined and were evaporated to approximately 10 mL under nitrogen. They were injected without additional processing. Separations were performed on a C18 column with an isocratic mobile phase of methanol/acetonitrile (60:40, volume fraction) containing 5 mmol/L ammonium acetate. The separation was monitored using an absorbance detector at 325 nm, but MS was used for quantitation. Retinyl palmitate and retinyl palmitate-d_4 were monitored at *m/z* 269 and *m/z* 273, respectively. Vitamins D_3 and D_3-d_3 were monitored at *m/z* 385 and *m/z* 388, respectively. Vitamins K_1 and K_1-d_6 were monitored at *m/z* 452 and *m/z* 458, respectively. Calibrants were prepared gravimetrically, with concentrations assigned spectrophotometrically, at levels intended to approximate the levels of the fat-soluble vitamins in the SRM following extraction. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Total α-Tocopherol and α-Tocopheryl Acetate Using LC-Absorbance and/or LC-Fluorescence: Mass fractions of vitamin E (as total α-tocopherol and α-tocopheryl acetate) were measured by LC-absorbance and/or LC-fluorescence from duplicate 1 g to 2 g test portions of SRM 1849a from each of 10 packets. Samples were accurately weighed into 50 mL polyethylene centrifuge tubes. An aliquot of an ethanolic tocol internal standard solution was added. The sample was suspended in approximately 5 mL of water and an aliquot of dipotassium oxalate solution was added. The analytes were then extracted from the suspended sample into a mixture of ethanol/*tert*-butylmethylether/petroleum ether (10:5:7, volume fractions) by rotational agitation for 15 min. Five additional extractions were performed using 15 min of mixing/rotation. The supernatants for the individual extractions were combined, washed with water twice, and were evaporated to dryness under nitrogen. The residues were resuspended in approximately 2 mL of ethanol/ethyl acetate (50:50, volume fraction). Separations were performed on a C18 column with an isocratic mobile phase of methanol/water (90:10, volume fraction). The separation was monitored using an absorbance detector at 295 nm for α-tocopherol acetate and a fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm for α-tocopherol. Calibrants were prepared gravimetrically, with concentrations assigned spectrophotometrically, at levels intended to approximate the levels of

the fat-soluble vitamins in the SRM following extraction. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Ascorbic Acid Using LC-Absorbance: The mass fraction of vitamin C (ascorbic acid) was measured by LC absorbance in duplicate 2 g test portions from each of 10 packets of SRM 1849a. Samples were dissolved in 30 g to 35 g of HPLC-grade water and 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. Separations were performed on a C18 column using a gradient LC method with potassium phosphate (dibasic)/acetonitrile mobile phase. The separation was monitored using an absorbance detector at 243 nm. Calibrants were prepared gravimetrically, at levels intended to approximate the level of ascorbic acid in the SRM following extraction. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Thiamine, Riboflavin, Niacinamide, Pantothenic Acid, and Pyridoxine Using ID-LC-MS: Mass fractions of vitamin B_1 (thiamine), vitamin B_3 (niacinamide), vitamin B_5 (pantothenic acid), and vitamin B_6 (pyridoxine) were measured by ID-LC-MS, and vitamin B_2 (riboflavin) were measured by LC-MS in duplicate 2 g test portions taken from each of 10 packets of SRM 1849a. Four internal standards were added: $^{13}C_3$-thiamine chloride; $^{2}H_4$-niacinamide; calcium $^{13}C_3$,^{15}N-pantothenate; and $^{13}C_4$-pyridoxine hydrochloride. The analytes and internal standards were extracted into dilute acetic acid for analysis by positive-ion MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C18 column were used for LC-MS determination. 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. 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. Riboflavin was measured at m/z 377, with $^{13}C_4$-pyridoxine as the internal standard. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the water-soluble vitamins in the SRM following extraction. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Folic Acid and 5-Methyltetrahydrofolate Using ID-LC-MS/MS: Mass fractions of folic acid and 5-methyltetrahydrofolate were measured by ID-LC-MS/MS on two 1.0 g test portions taken from each of 10 packets of SRM 1849a. Internal standards $^{13}C_5$-folic acid and $^{13}C_5$-5-methyltetrahydrofolate were added. A sodium phosphate buffer containing ascorbic acid was added and samples were subjected to trienzyme digestion with protease, α-amylase, and deconjugase [9]. Supernatants from centrifuged samples were filtered through 0.45 µm polyvinylidene difluoride (PVDF) filters and analyzed for 5-methyltetrahydrofolate by positive mode LC-MS/MS. Folic acid and $^{13}C_5$-folic acid were extracted on solid-phase extraction cartridges and eluted with a water/methanol solution containing ascorbic acid and formic acid for positive mode LC extraction cartridges and eluted with a water/methanol solution containing ascorbic acid and formic acid for positive mode LC-MS/MS analysis. A gradient LC method with a water/acetonitrile/formic acid mobile phase and a C18 column were used for the determination of both folic acid and 5-methyltetrahydrofolate. The transitions m/z 442.4 $\rightarrow$ m/z 295.1 and m/z 447.4 $\rightarrow$ m/z 295.1 were monitored for folic acid and $^{13}C_5$-folic acid, respectively. The transitions m/z 460.5 $\rightarrow$ m/z 176.1 and m/z 465.5 $\rightarrow$ m/z 176.1 were monitored for 5-methyltetrahydrofolate and $^{13}C_5$-5-methyltetrahydrofolate, respectively. Calibrants were prepared gravimetrically, with concentration assigned spectrophotometrically, at levels intended to approximate the levels of the folates in the SRM following extraction. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Biotin Using ID-LC-MS: The mass fraction of biotin was measured by ID-LC-MS in two 1.0 g test portions taken from each of 10 packets of SRM 1849a with $^{2}H_2$-biotin added as an internal standard. An aqueous formic acid solution was added to the samples, which were then subjected to mechanical shaking. Samples were centrifuged, and biotin and $^{2}H_2$-biotin were extracted on solid-phase extraction cartridges and eluted with a water/methanol solution containing formic acid for positive mode LC-MS analysis. An isocratic LC method with a water/methanol/formic acid mobile phase and a C18 column were used for the determination of biotin. Biotin and $^{2}H_2$-biotin were monitored at m/z 245 and m/z 247, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of biotin in the SRM following extraction. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Total Choline and Free Carnitine Using ID-LC-MS: Mass fractions of choline and carnitine were measured by ID-LC-MS in two 1.0 g test portions taken from each of 10 packets of SRM 1849a with $^{2}H_9$-choline chloride and $^{2}H_9$-carnitine hydrochloride added as internal standards. The analytes and internal standards were extracted and hydrolyzed by microwave digestion into dilute hydrochloric acid for analysis by positive-ion mode LC-MS. A gradient method with an ammonium formate/acetonitrile mobile phase and a mixed-mode C18 column were used for LC-MS determination. Choline and $^{2}H_9$-choline were measured at m/z 104 and m/z 113, respectively. Carnitine and $^{2}H_9$-carnitine were measured at m/z 162 and m/z 171, respectively. Calibrants were prepared

gravimetrically, at levels intended to approximate the levels of choline and carnitine in the SRM following extraction. 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 1849a was based on the combination of measurements made at NIST, collaborating laboratories, and the manufacturer, where available. NIST provided measurements by using inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), instrumental neutron activation analysis (INAA), and wavelength dispersive X-ray fluorescence spectrometry (WDXRF).

NIST Analyses for Ca, Cr, Cu, Fe, Mg, Mn, Mo, Na, P, K, Se, and Zn Using ICP-OES and/or ICP-MS: Mass fractions of calcium, chromium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, and zinc were measured by ICP-OES in duplicate 1.0 g test portions taken from each of 10 packets of SRM 1849a. Mass fractions of chromium, molybdenum, and selenium were measured by ICP-MS using duplicate 0.5 g test portions taken from each of 10 packets of SRM 1849a. Samples were digested using nitric acid or a nitric acid/hydrofluoric acid mixture in a microwave oven. Quantitation was based on the method of standard additions.

NIST Analyses for I Using ICP-MS: The mass fraction of iodine was measured by ICP-MS in duplicate 0.5 g test portions taken from each of six packets of SRM 1849a. Samples were digested using nitric acid in a microwave oven. After digestion, the pH was raised in the sample solutions by the addition of ammonium hydroxide. Quantitation was based on the method of standard additions.

NIST Analyses for Cl, I, and Mn Using INAA: Mass fractions of chlorine, iodine, and manganese were determined by INAA in individual disks prepared from single 0.2 g test portions taken from each of 10 packets of SRM 1849a. Samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually at 20 MW for 60 s. Nuclides were counted for 5 min after a 5 min decay, for 10 min following a 10 min decay, and for 20 min after a few hours decay.

NIST Analyses for Cl using WDXRF: The mass fraction of chlorine was determined using WDXRF analyses of briquettes prepared of duplicate, nominal 4.0 g test portions taken from each of six packets of SRM 1849a. To accurately quantify Cl, the elements Na, Mg, P, S, Cl, K, Ca, Mn, Fe, Cu, and Zn were analyzed by measuring the $K\text{-}L_{2,3}$ characteristic X-ray lines of all elements and calibrating with briquettes prepared from a suite of NIST SRMs for food and vegetable matter.

Collaborating Laboratories' Analyses: The GMA FIACC collaborating laboratories and several other laboratories were asked to use their usual methods to make measurements on single test portions taken from each of two packets of SRM 1849a. The manufacturer of the material also provided data for several nutrients.

Homogeneity Assessment: The homogeneity of elements, fatty acids, cholesterol, and vitamins was assessed at NIST using the methods and test portion sizes described in this certificate (see "Instructions for Storage and Use"); analysis of variance at a 5 % significance level did not show statistically significant heterogeneity. All analytes have been treated as though they are homogeneously distributed in the material although the homogeneity of the other analytes was not assessed.

Value Assignment: The GMA FIACC collaborating laboratories reported the individual results for each of their analyses for a given analyte and the mean of each laboratory's results was determined. For calculation of assigned values, the median of the individual GMA FIACC collaborating laboratory means, the manufacturer's mean, Hong Kong Government Laboratory's mean, and the mean of the individual sets of NIST data were averaged, as appropriate based on available data.

Certified Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST, the mean of the material manufacturer's data, and the median of the means of results provided by collaborating laboratories, where appropriate. 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 is believed to lie 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]. The measurand is the total mass fraction for each analyte listed in Table 1, on an as-received basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as grams per 100 grams).

Certified Mass Fraction Value for Cholesterol: The certified mass fraction for cholesterol is the mean of results obtained by NIST using ID-GC/MS. The value is 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 is believed to lie 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]. The measurand is the total mass fraction for cholesterol listed in Table 1, on an as-received basis. Metrological traceability is to the SI derived unit for mass fraction. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per gram).

Table 1. Certified Mass Fraction Values for Fatty Acids (as Free Fatty Acids) and Cholesterol in SRM 1849a

		Mass Fraction (g/100 g)		
Octanoic Acid (C8:0)[a,b,c]	Caprylic Acid	0.74	±	0.14
Hexadecanoic Acid (C16:0)[a,b]	Palmitic Acid	2.10	±	0.15
(Z)-9-Hexadecenoic Acid (C16:1 n-7)[a,b]	Palmitoleic Acid	0.0222	±	0.0042
Octadecanoic Acid (C18:0)[a,b]	Stearic Acid	0.809	±	0.046
(Z)-9-Octadecenoic Acid (C18:1 n-9)[a,b]	Oleic Acid	10.7	±	1.1
(Z)-11-Octadecenoic Acid (C18:1 n-7)[a,b]	Vaccenic Acid	0.196	±	0.023
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)[a,b,c]	Linoleic Acid	5.72	±	0.58
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)[a,b,c]	α-Linolenic Acid	0.591	±	0.081
Eicosanoic Acid (C20:0)[a,b]	Arachidic Acid	0.0822	±	0.0061
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6)[a,c]	Arachidonic Acid	0.123	±	0.011
Docosanoic Acid (C22:0)[a,b]	Behenic Acid	0.0660	±	0.0057
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6 n-3)[a,c]	DHA	0.0179	±	0.0024
Tetracosanoic Acid (C24:0)[a,b]	Lignoceric Acid	0.0387	±	0.0079
(Z)-15-Tetracosenoic Acid (C24:1 n-9)[a,b]	Nervonic Acid	0.0202	±	0.0022
Fat (as the sum of fatty acids as triglycerides)[a,b]		27.9	±	2.2
Saturated Fatty Acids[a,b]		9.42	±	0.63
Cis-Monounsaturated Fatty Acids[a,b]		11.1	±	1.2
Cis-Polyunsaturated Fatty Acids[a,b]		6.07	±	0.50
Omega-3 Fatty Acids[a,b]		0.568	±	0.027
Omega-6 Fatty Acids[a,b]		5.55	±	0.33
		Mass Fraction (mg/g)		
Cholesterol[d]		0.1374	±	0.0029

[a] NIST GC-FID
[b] Collaborating laboratories
[c] Manufacturer
[d] NIST ID-GC-MS

Certified Mass Fraction Values for Elements: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST, the mean of the material manufacturer's data, the mean of the results from Hong Kong Government Laboratory, and/or the median of the means of results provided by collaborating laboratories, where appropriate. 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 is believed to lie 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]. The measurand is the total mass fraction for each analyte listed in Table 2, on an as-received basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per per kilogram).

Table 2. Certified Mass Fraction Values for Elements in SRM 1849a

	Mass Fraction (mg/kg)		
Calcium (Ca)[a,b,c]	5253	±	51
Copper (Cu)[a,b,c]	19.78	±	0.26
Chlorine (Cl)[d,e,f]	7001	±	89
Chromium (Cr)[a,b,c,g]	1.072	±	0.032
Iodine (I)[b,g,h]	1.29	±	0.11
Iron (Fe)[a,b,c]	175.6	±	2.9
Magnesium (Mg)[a,b,c]	1648	±	36
Manganese (Mn)[a,b,h]	49.59	±	0.97
Molybdenum (Mo)[a,b,c,g]	1.707	±	0.040
Phosphorus (P)[a,b,c]	3990	±	140
Potassium (K)[a,b,c]	9220	±	110
Selenium (Se)[b,c,g]	0.812	±	0.029
Sodium (Na)[a,b,c]	4265	±	83
Zinc (Zn)[a,b,c]	151.0	±	5.6

[a] NIST ICP-OES
[b] Collaborating laboratories
[c] Manufacturer
[d] Hong Kong Government Laboratory
[e] NIST INAA
[f] NIST WDXRF
[g] NIST ICP-MS
[h] NIST INAA

Certified Mass Fraction Values for Vitamins and Carnitine: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST, the mean of the material manufacturer's data, and the median of the means of results provided by collaborating laboratories, where appropriate. 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 is believed to lie 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]. The measurand is the total mass fraction for each analyte listed in Table 3, on an as-received basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

Table 3. Certified Mass Fraction Values for Vitamins and Carnitine in SRM 1849a

	Mass Fraction (mg/kg)		
Ascorbic Acid (Vitamin C)[a,b,c]	784	±	65
Thiamine (Vitamin B_1)[c,d,e]	12.57	±	0.98
Riboflavin (Vitamin B_2)[c,f]	20.37	±	0.52
Niacinamide (Vitamin B_3)[c,d]	108	±	10
Pantothenic Acid (Vitamin B_5)[c,d]	68.2	±	1.9
Pyridoxine (Vitamin B_6)[c,d,g]	13.46	±	0.93
Folic Acid[b,c,h,i]	2.293	±	0.062
Biotin[b,c,d]	1.99	±	0.13
Choline Ion[b,c,d,j]	1090	±	110
Carnitine[b,c,d]	136	±	14
Retinol (Vitamin A)[b,c,d,i,k]	7.68	±	0.23
Retinyl Palmitate (Vitamin A)[c,d,i]	14.30	±	0.20
Cholecalciferol (Vitamin D_3)[b,c,d,i]	0.111	±	0.017
α-Tocopheryl Acetate (Vitamin E)[a,b,c,i]	158	±	18
Total α-Tocopherol (Vitamin E)[b,i,l,m]	219	±	16
Phylloquinone (Vitamin K_1)[b,c,d,i]	1.06	±	0.17

[a] NIST LC-absorbance
[b] Collaborating laboratories
[c] Manufacturer
[d] NIST ID-LC-MS
[e] Vitamin B_1 is reported as thiamine ion (265.36 g/mol), not thiamine chloride or thiamine chloride hydrochloride.
[f] NIST LC-MS
[g] Vitamin B_6 is reported as pyridoxine (169.18 g/mol), not pyridoxine hydrochloride.
[h] NIST ID-LC-MS/MS
[i] Metrological traceability is established through the molar absorptivity of the compound.
[j] Total choline, reported as the ion.
[k] Retinol was added to SRM 1849a as retinyl palmitate. NIST measured retinyl palmitate and converted the mass fraction to retinol equivalents by multiplying by the ratio of the relative molecular masses of retinol and retinyl palmitate. The certified value is expressed as retinol equivalents, and represents total (*cis* + *trans*) retinol. No correction is made for differences in biological activity of the *cis* and *trans* forms.
[l] NIST LC-fluorescence
[m] α-Tocopherol was added to SRM 1849a 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-tocopheryl acetate that was added.

Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each reference mass fraction value is the combined mean from the mean of NIST GC-FID data and the median of the mean results provided by collaborating laboratories, where available. Values are expressed as $x \pm U_{95\%}(x)$, where x is the reference value and $U_{95\%}(x)$ is the expanded uncertainty of the value at a confidence level of approximately 95 %. The measurand is the mass fraction for each analyte listed in Table 4, on an as-received basis, as determined by the method indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams) as realized by the method used.

Table 4. Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids in SRM 1849a

		Mass Fraction (g/100 g)		
Hexanoic Acid (C6:0)[a]	Caproic Acid	0.0625	±	0.0099
Decanoic Acid (C10:0)[a,b]	Capric Acid	0.57	±	0.14
Dodecanoic Acid (C12:0)[b]	Lauric Acid	3.99	±	0.28
Tetradecanoic Acid (C14:0)[b]	Myristic Acid	1.476	±	0.075
Heptadecanoic Acid (C17:0)[a]	Margaric Acid	0.0140	±	0.0021
Total Trans C18:1 Fatty Acids[a]		0.0242	±	0.0050
(Z,E)-9,12-Octadecadienoic Acid (C18:2)[a]		0.0205	±	0.0032
(E,Z)-9,12-Octadecadienoic Acid (C18:2)[a]		0.0186	±	0.0029
(Z,Z,Z)-6,9,12-Octadecatrienoic Acid (C18:3 n-6)[a]		0.0300	±	0.0020
(Z)-11-Eicosenoic Acid (C20:1 n-9)[a,b]	Gondoic Acid	0.069	±	0.017
Total Trans Fatty Acids[a]		0.085	±	0.022

[a] Collaborating laboratories
[b] NIST GC-FID

Reference Values for Proximates, Lactose Monohydrate, and Calories: Each reference mass fraction value is the combined mean from the mean of the results provided by the material manufacturer and the median of the mean results provided by collaborating laboratories, where available. Values are expressed as $x \pm U_{95\%}(x)$, where x is the reference value and $U_{95\%}(x)$ is the expanded uncertainty of the value at a confidence level of approximately 95 %. For proximates and lactose monohydrate, the measurands are the mass fractions listed in Table 5, on an as-received 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 an as-received basis as determined by the method indicated, and metrological traceability is to the scale realized by that method for energy.

Table 5. Reference Values for Proximates, Lactose Monohydrate, and Calories in SRM 1849a

	Mass Fraction (g/100 g)		
Solids[(a)]	98.28	±	0.15
Ash[(a,b)]	4.695	±	0.020
Fat (extracted)[(a,b)]	30.43	±	0.95
Protein[(a,b,c)]	13.225	±	0.056
Carbohydrates[(a)]	51.6	±	1.3
Lactose Monohydrate[(a)]	47.6	±	5.5
	Energy (kcal per 100 g)		
Calories[(d)]	520.8	±	6.4

[(a)] Collaborating laboratories
[(b)] Manufacturer
[(c)] Results for nitrogen were converted to protein using a factor of 6.38.
[(d)] 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 521.2 kcal/100 g.

Reference Mass Fraction Values for Vitamins and *myo*-Inositol: Each reference mass fraction value is the combined mean from the mean of NIST data, the mean of the material manufacturer's data, the mean of the results from Hong Kong Government Laboratory, or the median of the mean results provided by collaborating laboratories, where available. Values are expressed as $x \pm U_{95\%}(x)$, where x is the reference value and $U_{95\%}(x)$ is the expanded uncertainty of the value at a confidence level of approximately 95 %. The measurand is the mass fraction for each analyte listed in Table 6, on an as-received basis, as determined by the method indicated. Metrological traceability is to mass fraction (expressed as milligrams per kilogram) as realized by the method used.

Table 6. Reference Mass Fraction Values for Vitamins and *myo*-Inositol in SRM 1849a

	Mass Fraction (mg/kg)		
5-Methyltetrahydrofolate[(a,b)]	0.0839	±	0.0031
Vitamin B_{12}[(c,d)]	0.0482	±	0.0085
Free α-Tocopherol[(b,e)]	89.2	±	1.9
myo-Inositol[(d,f)]	405.2	±	7.6

[(a)] NIST ID-LC-MS/MS
[(b)] Metrological traceability is established through the molar absorptivity of the compound
[(c)] Collaborating laboratories
[(d)] Manufacturer
[(e)] NIST LC-fluorescence
[(f)] Hong Kong Government Laboratory

Reference Mass Fraction Values for Amino Acids and Taurine: Each reference mass fraction value is the combined mean from the mean of the material manufacturer's data and the median of the mean results provided by collaborating laboratories, where available. Values are expressed as $x \pm U_{95\%}(x)$, where x is the reference value and $U_{95\%}(x)$ is the expanded uncertainty of the value at a confidence level of approximately 95 %. The measurand is the mass fraction for each analyte listed in Table 7, on an as-received basis, as determined by the method indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams) as realized by the method used.

Table 7. Reference Mass Fraction Values for Amino Acids and Taurine in SRM 1849a

	Mass Fraction (g/100 g)		
Alanine[a]	0.455	±	0.021
Arginine[a]	0.400	±	0.029
Aspartic Acid[a]	1.070	±	0.057
Cystine[a,b]	0.1286	±	0.0071
Glutamic Acid[a]	2.59	±	0.27
Glycine[a]	0.241	±	0.019
Histidine[a]	0.315	±	0.036
Isoleucine[a]	0.660	±	0.071
Leucine[a]	1.261	±	0.050
Lysine[a]	1.010	±	0.071
Total Methionine[a,b]	0.482	±	0.051
Phenylalanine[a]	0.580	±	0.021
Proline[a]	1.195	±	0.086
Serine[a]	0.720	±	0.030
Taurine[a,b]	0.0366	±	0.0018
Threonine[a]	0.640	±	0.022
Tryptophan[a]	0.184	±	0.010
Tyrosine[a]	0.510	±	0.043
Valine[a]	0.76	±	0.11

[a] Collaborating laboratories
[b] Manufacturer

Reference Mass Fraction Values for Nucleotides: Each reference mass fraction value is the median of the mean results provided by collaborating laboratories. Values are expressed as $x \pm U_{95\%}(x)$, where x is the reference value and $U_{95\%}(x)$ is the expanded uncertainty of the value at a confidence level of approximately 95 %. The measurand is the mass fraction for each analyte listed in Table 8, on an as-received basis, as determined by the method indicated. Metrological traceability is to mass fraction (expressed as milligrams per kilograms) as realized by the method used.

Table 8. Reference Mass Fraction Values for Nucleotides in SRM 1849a

	Mass Fraction (mg/kg)		
Adenosine Monophosphate	105.1	±	5.3
Cytidine Monophosphate	268	±	29
Guanosine Monophosphate	146	±	11
Uridine Monophosphate	129	±	15

Information Mass Fraction Values for Other Measurands: Each information mass fraction value is the mean of approximately 30 measurements provided by the manufacturer. Information values cannot be used to establish metrological traceability.

Table 9. Information Mass Fraction Values for Other Measurands in SRM 1849a

	Mass Fraction (mg/kg)
Free Choline Ion	798
Adenosine Monophosphate + Adenosine[a]	108
Cytidine Monophosphate + Cytidine[a]	317
Guanosine Monophosphate + Guanosine[a]	146
Uridine Monophosphate + Uridine[a]	155

[a] The mass fraction value represents the sum of the nucleotide and the nucleoside calculated as the nucleotide.

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Certificate Revision History: 13 July 2018 (Chlorine reference value updated to certified value using NIST WDXRF data and moved from Table 6 to Table 2; added reference value for total methionine; editorial changes); **29 October 2015** (Expiration date changed; Fatty acid values updated to certified values using collaborating laboratories data and NIST data and moved from Table 4 to Table 1; values for summed fatty acids updated from reference to certified values based on inclusion of NIST data and moved from Table 5 to Table 1; editorial changes); **15 June 2015** (Corrects footnote for Riboflavin in Table 3; editorial changes); **19 December 2014** (Corrects mean caloric content value listed in the footnote for Table 5; editorial changes); **16 October 2014** (Certified fatty acid values changed to reference values and moved from Table 1 to Table 4, fatty acid values only include collaborating laboratories data; updated protein value in Table 5; corrected niacinamide value in Table 3; changed footnotes in Table 3; editorial changes); **29 April 2014** (Updated a fatty acid name in Table 4; editorial changes); **17 January 2014** (Certified values added for α-tocopheryl acetate and retinyl palmitate; reference value added for free α-tocopherol; total α-tocopherol value updated using a NIST method; chlorine and myo-inositol values updated using a second method; footnotes added to Table 3 to clarify the forms of thiamine and pyridoxine; editorial changes); **07 August 2012** (Certified value added for iodine; manganese value updated using a third method; information value added for chlorine; collaborating laboratories removed from appendix and listed in the certificate body; footnote added to Table 3 to clarify the form of choline; editorial changes); **05 April 2012** (Correction of the names for Vitamin B_1 and B_6 in Table 3 to indicate the base form used for listed values; editorial changes); **30 January 2012** (Corrected alternate name for eicosanoic acid in Table 1; editorial changes); **01 December 2011** (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 https://www.nist.gov/srm.