National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1548a

Typical Diet

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of proximate content and minor and trace constituent elements in mixed diets and food matrices. The base material used for SRM 1548a originated from food menus used for metabolic studies performed at the USDA Beltsville Human Nutrition Research Center. SRM 1548a is a blended freeze-dried homogenate of these menus. This SRM may also be used for quality assurance when assigning values to in-house control materials. SRM 1548a Typical Diet is the renewal for SRM 1548 Total Diet. A unit of SRM 1548a consists of two bottles, each containing approximately 6.5 g of the freeze-dried homogenate of mixed diet foods.

Certified Mass Fraction Values: The certified mass fraction values for the minor elements and several trace elements 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]. Values were derived from the combination of results provided by NIST and collaborating laboratories (Appendices A, B, and C). Values are reported on a dry mass basis in mass fraction units [2].

Reference Mass Fraction Values: Reference mass fraction values for four trace elements (Table 3), proximates and calories (Table 4), which were derived from results reported by collaborating laboratories. The reference values are noncertified values that do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple methods. Explanations in support of each reference value are given as a note in each table. Values are reported on a dry-mass basis in mass fraction units.

Information Mass Fraction Values: Information mass fraction values for additional trace elements are provided in Table 5. These are noncertified values with no uncertainties reported as there is insufficient information to make an assessment of the uncertainties. The information values are given to provide additional characterization of the material. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 1548a** is valid, within the measurement uncertainty specified, until **30 April 2026**, 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 technical measurements leading to certification of this SRM were performed by R.R. Greenberg of the NIST Chemical Sciences Division and G.V. lyengar of the International Atomic Energy Agency.

Coordination of the material preparation was under the direction of W.R. Wolf of the U.S. Department of Agriculture (USDA), Beltsville, MD.

Analytical measurements at NIST were performed by D.A. Becker, R. Demiralp, K. Garrity, R.R. Greenberg, G.V. Iyengar, R.M. Lindstrom, S.E. Long, E.A. Mackey, B.R. Norman, R.L. Paul, M.S. Rearick, L. Tandon, G.C. Turk, and L.J. Wood of the NIST Chemical Sciences Division.

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Analytical measurements for value assignment were performed by NIST and the following collaborating laboratories: the Centers for Disease Control and Prevention/Nutrition Biochemistry Branch, Atlanta, GA; Food and Drug Administration/Atlanta Center for Nutrient Analysis, Atlanta, GA; Perkin-Elmer Corporation, Norwalk, CT; USDA Beltsville Human Nutrition Research Center/Food Composition Laboratory and Vitamin and Mineral Nutrition Laboratory, Beltsville, MD; University of Ljubljana/Laboratory for Radiochemistry, Ljubljana, Slovenia; University of Massachusetts at Amherst/Department of Chemistry, Amherst, MA; and Virginia Polytechnic and State University/Department of Biochemistry and Anaerobic Microbiology, Blacksburg, VA. In addition, the following three contract laboratories provided confirmatory analysis for proximate contents: Covance Laboratories, Inc. (formerly Corning Hazleton) Madison, WI, Lancaster Laboratories, Lancaster, PA, and Medallion Laboratories, Minneapolis, MN.

Statistical analysis was provided by L.M. Gill 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 1548a IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: SRM 1548a should be stored under refrigeration at a temperature between 2 °C and 8 °C in its original container and tightly capped. It should **NOT** be exposed to intense direct light or ultraviolet radiation.

Use: Allow the bottle to come to ambient temperature before opening. Exposure of sample to air should be minimized and any unused portion stored as described under "*Storage*". Before each use, the contents of the bottle should be well mixed by gently shaking and rolling the container. The recommended minimum sample mass for analysis of this SRM is 250 mg for most minor and trace elements. Residual moisture content should be determined on a separate sample for conversion of analytical results to a dry mass basis. The recommended drying method is freeze-drying. If freeze-drying is not available, the sample should be vacuum dried at a temperature not to exceed 25 °C for 24 h. The observed moisture content ranges from 1 % to 15 % (mass fraction). Due to high fat content of this material, excessive drying times and elevated temperatures will lead to loss of volatile lipid components and an incorrect estimation of dry mass.

PREPARATION AND ANALYSIS

Preparation: This SRM was prepared from menus used for the metabolic studies at the Human Study Facility of the USDA Beltsville Human Nutrition Research Center. Food items in prescribed quantities representing a four-day menu cycle were pooled/combined into a master menu. Enough foods were acquired to prepare a sufficient quantity of the diet composite. The material was freeze-dried, pulverized, sieved, and radiation sterilized at a dose of 2.5 mrad to prevent bacterial growth. The resulting product was blended, bottled, and sealed under nitrogen.

Homogeneity: Due to the substantial fat content in this pooled material and the simplified processing steps adopted during the preparation, the final product is not a free flowing powder. Nevertheless, for major matrix components (e.g., fat and protein), the blend is homogeneous. The degree of homogeneity observed for elements at different concentration levels, corresponds to what is practically attainable for such multicomponent solid mixtures and, therefore, the material is also considered to be sufficiently homogeneous for minor and trace elements.

NIST Analyses for Minor and Trace Elements

NIST analyses were performed by neutron activation analysis and mass spectrometry using appropriate combinations as shown in Appendix C. At least six independent determinations were made for each analyte. With the exception of phosphorus (two determinations each from three bottles) the remaining assays represent results obtained by analyzing 6 to 12 bottles for all other analytes. The amount of sample used for each determination varied depending upon the method of analysis. In most cases, the sample masses ranged from 0.25 g to 1 g, with the exception of cadmium and lead by isotope dilution mass spectrometry where 1 g to 2 g samples were used, and iodine by neutron activation analysis following pre-irradiation combustion separation where up to 2.5 g samples were used.

Collaborating Laboratories' Analyses for Proximate Constituents and Minor and Trace Elements

Proximate contents (ash, fat, nitrogen/protein, and solids) were determined by several laboratories (see Appendix A and Appendix B). At least six independent determinations were made for each analyte, with the exception of two contract laboratories, which carried out two determinations each from three bottles. The amount of sample used varied from 1 g to 3 g depending upon the analyte. AOAC Official Methods [3] were used for the determination of nitrogen, fat, ash, and solids as listed in Appendix B.

For the determination of minor and trace elements, flame atomic absorption spectrophotometry, graphite furnace atomic emission spectrophotometry, inductively coupled plasma mass spectrometry, inductively coupled plasma spectrometry, and neutron activation analysis (radiochemical) were used by various laboratories. At least six independent determinations were made for each analyte. The amount of sample used varied from 0.25 g to 0.5 g depending upon the method used, with the exception of one laboratory that used 2.5 g to prepare the matrix stock solution for assay by multiple modes of a given technique.

Value Assignment: The participating laboratories reported a minimum of six determinations based on six separate measurements (with two exceptions as mentioned under analyses carried out at NIST and collaborating laboratories). All laboratories used specified controls to validate the methods and reported their results for such control material analysis. All assigned values are the equally weighted means of the measurements for a given analyte made by NIST and by collaborating laboratories reporting results. The associated uncertainties are expanded at the 95 % level of confidence with a bias adjustment for differences between methods [1,4].

Table 1. Certified Mass Fraction Values for Minor Elements[a]

Element	Mass Fraction (mg/kg)		
Calcium	1967	±	113
Chlorine	12078	±	356
Magnesium	580	±	27
Phosphorus	3486	±	245
Potassium	6970	±	125
Sodium	8132	±	942
Sulfur	1928	±	150

[a] Each certified mass fraction value is an equally weighted mean from the combination of results provided by NIST and collaborating laboratories. The uncertainty in the certified mass fraction values is expressed as an expanded uncertainty, U, which is calculated as, $U = ku_c + B$. The combined standard uncertainty, u_c, is calculated according to the ISO/JCGM Guides [1], which accounts for the combined effect of the within variance for all participating laboratories at one standard deviation. The coverage, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and method means [4]. The measurand is the total mass fraction value for each analyte listed. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

Table 2. Certified Mass Fraction Values for Trace Elements[a]

Element	Mass Fraction (mg/kg)		
Aluminum	72.4	±	1.52
Arsenic[b]	0.20	±	0.01
Cadmium	0.035	±	0.0015
Cesium	0.0098	±	0.0003
Copper	2.32	±	0.16
Iron	35.3	±	3.77
Iodine	0.759	±	0.103
Manganese	5.75	±	0.17
Nickel	0.369	±	0.023
Lead	0.044	±	0.009
Selenium	0.245	±	0.028
Tin	17.2	±	2.57
Zinc	24.6	±	1.79

[a] Each certified mass fraction value (with the exception of the one for arsenic) is an equally weighted mean from the combination of results provided by NIST and collaborating laboratories. The uncertainty in the certified mass fraction values is expressed as an expanded uncertainty, U, which is calculated as $U = ku_c + B$. The combined standard uncertainty, u_c, is calculated according to the ISO/JCGM Guides [1], which accounts for the combined effect of the within variance for all participating laboratories at one standard deviation. The coverage, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and method means [4]. The measurand is the total mass fraction value for each analyte listed. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

[b] The certified value for arsenic is based on NIST measurements and its uncertainty has been expanded by a coverage factor of $k = 4$. The measurand is the certified mass fraction value for arsenic. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

The mass fraction values in Table 3 are provided as reference values because the results were not confirmed by an independent analytical technique or a definitive method as required for NIST certification.

Table 3. Reference Mass Fraction Values for Trace Elements[a]

Element	Mass Fraction[a] (mg/kg)		
Boron[b]	4.16	±	0.04
Barium	1.1	±	0.10
Molybdenum	0.260	±	0.017
Strontium	2.93	±	0.10

[a] Each reference mass fraction value is an equally weighted mean from the combination of results provided by NIST and collaborating laboratories. The uncertainty in the reference mass fraction values is expressed as an expanded uncertainty, U, which is calculated as $U = ku_c + B$. The combined standard uncertainty, u_c is calculated according to the ISO/JCGM Guides [1], which accounts for the combined effect of the within variance for all participating laboratories at one standard deviation. The coverage, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte listed. B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and method means [4]. The measurand is the total mass fraction value for the elements based on the methods used by NIST and the collaborating laboratories. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

[b] The measurand is the reference mass fraction value for boron based on NIST measurements only. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

The mass fraction values in Table 4 are provided as reference values because the results have not been confirmed by an independent analytical technique or by a definitive method as required for NIST certification. These reference values should be useful for comparison with results obtained using similar procedures (i.e., AOAC methods).

Table 4. Reference Mass Fraction Values for Proximates and Calories

Analyte	Mass Fraction[a] (%)		
Ash	4.042	±	0.164
Carbohydrate[b]	58.36	±	1.53
Fat (total)	19.41	±	1.45
Nitrogen	3.03	±	0.31
Protein[c]	18.08	±	0.42
Total Dietary Fiber	4.31	±	0.21
Calories[d]	481.0	±	8.11 kcal/100 g

[a] Each matrix component reference value, expressed as a mass fraction of the material (dry mass basis) is an equally weighted mean from the combination of results provided by NIST (for nitrogen), collaborating laboratories and contract laboratories listed in Appendices A and B, respectively. The uncertainty in the reference value is expressed as an expanded uncertainty, U, which is calculated as $U = ku_c + B$. The combined standard uncertainty, u_c is calculated according to the ISO and NIST Guides [1], which accounts for the combined effect of the within variance for all participating laboratories at one standard deviation. The coverage, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and method means [4]. The measurand is the reference mass fraction values for each analyte listed based on an independent analytical technique or by a definitive method. Metrological traceability is to the SI derived unit for mass fraction (expressed as a percent).

[b] Calculated by difference.

[c] Calculated: a factor of 6.25 was used to convert nitrogen to protein.

[d] The measurand is the reference mass fraction value for calories is based on the mean of individual calculations from the laboratories reporting results. Metrological traceability is to the SI derived unit for calories (expressed as kilocalories be gram). If the mean proximate content values are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, carbohydrate, and protein, respectively, the mean caloric content is 480.5 kcal/100 g.

For Methods see Appendix B.

The mass fraction values in Table 5 are provided as information values due to a larger than expected variability of results among laboratories and/or are derived from analyses performed by a single method.

Table 5. Information Mass Fraction Values for Trace Elements

Analyte	Mass Fraction (mg/kg)
Antimony	0.009
Bromine	9.64
Cobalt	0.028
Mercury	0.005
Silicon	78.7
Scandium	0.0008
Titanium	4.7

REFERENCES

[1] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Oct 2020); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed Oct 2020).

[2] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/pml/special-publication-811 (accessed Oct 2020).

[3] AOAC International Official Methods of Analysis, 16th ed., AOAC International: Gaithersburg, MD (1995).

[4] Schiller, S.B.; Eberhardt, K.E.; *Combining Data from Independent Analysis Methods*; Spectrochimica Acta, Vol. 46B, No. 12, pp. 1607–1613 (1991).

[5] Official Methods of Analysis, 15th ed., AOAC International: Gaithersburg, MD (1990).

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.

APPENDIX A

Measurements by Collaborating Laboratories

Laboratory/Analyst(s)	Method(s)	Analytes
Centers for Disease Control and Prevention, Division of Environmental Laboratory Health Science; Atlanta, GA D.C. Paschal	Graphite Furnace Atomic Absorption Spectrophotometry, Inductively Coupled Plasma Mass Spectrometry	Cd, Ni, Pb Al, Cd, Ni, Pb
Food and Drug Administration Atlanta Center for Nutrient Analysis; Atlanta, GA K. Dodson	AOAC Official Methods (See Appendix B) Kjeldahl	Proximate content N
Perkin-Elmer Corporation; Norwalk, CT K. Barnes	Inductively Coupled Plasma Atomic Emission Spectrophotometry	Al, B, Ba, Ca, K, Mn, Na, Ni, P, S, Sn, Zn
USDA Beltsville Human Nutrition Research Center; Beltsville, MD Food Composition Laboratory N. Miller-Ihli B. Li	AOAC Official Methods[a] Flame Atomic Absorption Spectrophotometry	Total Dietary Fiber Ca, Cu, Fe, K, Mg, Mn, Na, Zn
Vitamin and Mineral Laboratory C. Veillon C. Patterson	Flame Atomic Absorption Spectrophotometry	Cu, Fe, Zn
University of Ljubljana Radiochemistry Laboratory, Ljubljana, Slovenia A.R. Byrne	Radiochemical Neutron Activation Analysis	I
University of Massachusetts Department of Chemistry; Amherst, MA R. Barnes A. Krushevska	Inductively Coupled Plasma Mass Spectrometry Inductively Coupled Plasma Atomic Emission Spectrophotometry	Al, As, Ba, Cs, Cu, Mn, Mo, Se, Sn, Sr, Zn Al, Ba, Ca, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, Zn
Virginia Polytechnic and State University, Department of Biochemistry; Blacksburg, VA K.M. Phillips	AOAC Official Methods (See Appendix B)	Proximate content

[a] AOAC Method 991.43 [5]

Measurements by Contract Laboratories using AOAC Official Methods of Analysis

Laboratory/Analyst(s)	Method(s)	Analytes
Covance Laboratories Inc. (formerly Corning Hazelton Inc.,); Madison, WI	Kjeldahl Extraction Muffle Furnace Vacuum Drying	Nitrogen/Protein [a] Total Fat [b] Ash [c] Solids [d] Total Dietary Fiber [e]
Lancaster Laboratories; Lancaster, PA	Kjeldahl Extraction Muffle Furnace Vacuum Drying	Nitrogen/Protein [a] Total fat [b] Ash [c] Solids [d]

[a] AOAC Methods 2.4.03 and 32.2.03 [2]
[b] AOAC Methods 922.06, 954.02 [5]
[c] AOAC Methods 32.1.05 [2]
[d] Solids (Following moisture determination by vacuum drying for 24 h at 25 °C)
[e] TDF AOAC Method 985.29 [5]

APPENDIX C

NIST Measurements, Analytical Chemistry Division

Method(s)	**Analytes**
Isotope Dilution Mass Spectrometry using Inductively Coupled Plasma-MS	Cd, Mg, Pb, Ni
Inductively Coupled Plasma-MS	Mo
Instrumental Neutron Activation	Al, Ca, Cl, Co, Cr, Cs, Fe, K, Mg, Na, Sc, Se, Sn, Zn
Prompt Gamma-Ray Activation	B, Cl, K, N, Na, S
Pre-concentration Neutron Activation Analysis	I
Radiochemical Neutron Activation	As, Cd, Cu, Hg, Sb, Se, Sn, P