



Certificate of Analysis

Standard Reference Material[®] 2910b

Hydroxyapatite

This Standard Reference Material (SRM) is intended for use in evaluating the physical and chemical properties of apatites with biological, geological or synthetic origins. The calcium to phosphorus (Ca/P) molar ratio for SRM 2910b is consistent with the theoretical Ca/P molar ratio of 1.67 for hydroxyapatite and a compositional formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. A unit of SRM 2910b consists of a 2 g bottle of hydroxyapatite powder.

Certified Mass Fraction Values: Certified values for constituents of SRM 2910b are reported in Table 1 as mass fractions (expressed as a percent) and Table 2 as Ca/P molar ratios on an as-received basis. 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]. A certified value is the present best estimate of the true value based on the results of analyses performed at NIST.

Information Values: Information values for X-ray Diffraction (XRD) peak positions are reported in Table 3, lattice parameters in Table 4, and carbonate content. An information value is considered to be a value that may be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value [1]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 2910b** is valid, within the measurement uncertainty specified, until **30 September 2025**, provided the SRM is handled and stored in accordance with the instructions given in the certificate (see "Instructions for Handling, 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 certification, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical activities required for certification of this SRM were performed by S. Lin-Gibson and D. Wang of the NIST Biosystems and Biomaterials Division.

The SRM was prepared by S. Frukhtbeyn of the Volpe Research Center, American Dental Association Foundation (ADAF), assigned to the NIST Biosystems and Biomaterials Division.

Certification of this SRM was performed by D. Wang and J.L. Molloy of the NIST Chemical Sciences Division.

Statistical consultation was provided by A.L. Pintar of the NIST Statistical Engineering Division.

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

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Robert L. Watters, Jr., Director
Office of Reference Materials

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Storage: The material should be kept in its original bottle, tightly closed, and stored in a desiccator.

Handling and Use: A minimum sample mass of 500 mg of material is necessary for values in this certificate to be valid within stated uncertainties. The contents of the bottle should be shaken well before each use, the bottle closed tightly immediately after use and stored as described above.

PREPARATION, ANALYSIS AND CERTIFIED VALUES

Preparation of Materials: The hydroxyapatite powder was produced at NIST in collaboration with the ADAF Volpe Research Center. The powder was synthesized by the solution reaction of calcium hydroxide and phosphoric acid [2]. In brief, approximately 2.5 L of distilled water was boiled for 60 minutes in a 5 L three-neck round bottom flask equipped with an overhead stirrer, reflux condenser coupled to a CO₂-absorbing NaOH trap, and ports for introducing titrant and nitrogen gas (purified by passing through 0.01 M KOH solution). Approximately 120 g of calcium oxide (prepared from calcium carbonate by heating at 1100 °C for 24 h) was added to the water and mixed vigorously. Phosphoric acid (0.5 mol·L⁻¹) was then added to the calcium oxide/calcium hydroxide slurry at a rate of 0.6 mL/min and to a final calculated Ca/P molar ratio of 1.67 (approximately 2.5 L of phosphoric acid). The mixture was refluxed for 2 days. After refluxing, the solution was cooled and the precipitated solid phase settled, the supernatant decanted, and an equal volume of boiled distilled water was added and further refluxed for another 2 days. This washing and boiling procedure was repeated four times until the pH of the supernatant was >6 which was the condition where residue traces of dicalcium phosphate were converted into hydroxyapatite. Six individual batches were synthesized, totaling approximately 1 kg of hydroxyapatite. The batches were combined in 3 L of deionized carbonate free water under boiling conditions and nitrogen atmosphere and mixed with an overhead stirrer for 5 days. The final precipitate was dried at 105 °C under vacuum for 5 days.

Certified Mass Fraction Values: The measurand is the total mass fraction for each constituent listed in Table 1. The certified values are metrologically traceable to the SI derived unit for mass fraction, expressed as percent. The calcium and phosphorous content were determined by X-ray fluorescence (XRF) and inductively coupled plasma optical emission spectroscopy (ICP-OES). Twenty four measurements were performed by XRF on twelve units of SRM 2910b and twelve measurements were performed by ICP-OES on six units of SRM 2910b. Each certified value is the estimate of the mean of a random effects model fitted to the data from the two methods. The estimates and associated uncertainties are calculated using the Bayesian inference paradigm [3]. The uncertainty listed with each certified value is an interval calculated in a consistent manner with the ISO/JCGM Guides [4,5], and it expresses contributions from all recognized sources of uncertainty, including differences among analytical methods, differences among bottles, dispersion of values resulting from sample preparation and replicated measurement, preparation and measurement of calibrants, analytical calibration functions, assay of primary materials, and balance calibration. The nominal coverage for each interval is 95 %. The measurand is the certified Ca/P molar ratio presented in Table 2 and is derived from the determinations of calcium and phosphorus. The certified values are metrologically traceable to the SI derived unit for mass, expressed as the ratio of the mass fractions.

Table 1. Certified Mass Fraction Values

Constituent	Mass Fraction (%)	95 % Coverage Interval
Calcium (Ca)	39.24	38.60 to 39.75
Phosphorus (P)	18.09	17.75 to 18.39

Table 2. Certified Ca/P Molar Ratio

Constituent	Molar Ratio	95 % Coverage Interval
Ca/P	1.676	1.639 to 1.714

Information Values: The following analysis was carried out to provide values for X-ray diffraction measurements and carbonate content to demonstrate that SRM 2910b is consistent the mineral hydroxyapatite. The values reported herein are considered for information only.

X-Ray Diffraction: The diffraction Instrumental analysis was performed on a Rigaku SmartLab⁽¹⁾. The XRD was equipped with a Cu rotating anode system, operated at 45 kV, 200 mA, producing CuK α radiation with diffracted-beam graphite monochromator. The instrument was configured to use a Bragg-Brentano geometry. A scintillation detector was used. The incident slit was set to 1 mm with 1 mm and 0.15 mm receiving slits of RS1 and RS2, respectively. Data were collected with a step width of 0.01° 2 θ and a count time of 4 seconds per step. The data analysis was performed using PowderX, a freely available software package [6].

Table 3. Information values for peak positions of SRM 2910b using CuK α Radiation, $\lambda = 0.154\ 059\ 3\ \text{nm}$

<i>hkl</i>	<i>2</i> θ	<i>d</i> (nm)	I (relative)
100	10.83	0.8160	10
101	16.83	0.5264	3
110	18.82	0.4711	2
200	21.77	0.4080	6
111	22.85	0.3889	5
201	25.35	0.3511	2
002	25.85	0.3444	34
102	28.10	0.3173	8
210	28.93	0.3084	15
211	31.76	0.2815	100
112	32.17	0.2780	48
300	32.90	0.2720	60
202	34.04	0.2632	21
301	35.45	0.2530	4
212	39.18	0.2298	6
310	39.80	0.2263	22
221	40.44	0.2229	2
311	41.99	0.2150	6
302	42.31	0.2135	1
113	43.83	0.2064	5
400	44.37	0.2040	1
203	45.28	0.2001	4
222	46.68	0.1944	28
312	48.07	0.1891	20
320	48.59	0.1872	4
213	49.45	0.1842	30
321	50.48	0.1807	15
410	51.26	0.1781	11
402	52.06	0.1755	12
004	53.14	0.1722	13
104	54.41	0.1685	1
322	55.85	0.1645	6
313	57.10	0.1612	4
501	58.03	0.1588	2
412	58.28	0.1582	1
330	58.75	0.1570	1
420	59.93	0.1542	4

⁽¹⁾ Certain commercial equipment, instrumentation, or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institutes of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 4. Information values for lattice parameters of SRM 2910b

	Lattice Parameter (nm)
ab	0.94227
c	0.68886

Carbonate Analysis: Carbonate content in candidate SRM 2910b was tested by Galbraith Laboratories (Knoxville, TN) using a Carbonate as Carbon quantitative method [7,8]. The analytical method liberates carbon dioxide from the sample through acidification and heat. Carbon dioxide-free nitrogen sweeps the evolved carbon dioxide through a scrubber and into an absorption cell, which automatically titrates coulometrically. This method determines carbon dioxide, carbonic acid, bicarbonate ion, and carbonate ion in a variety of materials, including aqueous, non-aqueous liquids and solids. The values are provided as mass fractions for carbon and can be converted to mass fractions carbonate by multiplying by ratio of the molecular mass of carbonate to the atomic mass of carbon (4.996). The carbonate content as measured is below the limit of quantitation (0.40 %).

REFERENCES

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Certificate Revision History: 16 December 2015 (Corrected methods for antimony and bismuth; editorial changes); 04 September 2015 (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>.