National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3950

Vitamin B_6 in Frozen Human Serum

This Standard Reference Material (SRM) is intended primarily for use in evaluating the accuracy of procedures for the determination of the vitamin B_6 metabolite pyridoxal 5'-phosphate (PLP) in human serum. It is also intended for use in validating working or secondary reference materials. PLP is the major circulating form of vitamin B_6 and the most common direct measure of this vitamin in serum or plasma. A unit of SRM 3950 consists of two stoppered vials of frozen human serum, one vial each at two different concentration levels. Each vial contains 1.0 mL of human serum.

The development of SRM 3950 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health (NIH), Office of Dietary Supplements (ODS). Analyses for value assignment were performed by NIST and the Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Certified Concentration Values: The certified concentration values for PLP are provided in Table 1. 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]. The certified concentration values for each level are based on the agreement of results from isotope dilution liquid chromatography/tandem mass spectrometry (ID LC-MS/MS) at NIST [2] and liquid chromatography/fluorescence detection (LC/FD) at the CDC [3]. All values were combined without weighting. The certified concentrations apply only to serum thawed to room temperature, 20 ºC to 25 ºC (see "Instructions for Storage and Use.")

Information Concentration Values: Information concentration values are provided for the vitamin B_6 metabolite 4-pyridoxic acid in Table 2. 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 [4]. Measurement of 4-pyridoxic acid was performed by LC/FD [3] at the CDC. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 3950** is valid, within the measurement uncertainty specified, until **31 March 2023**, 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.

Overall direction and coordination of the technical activities were performed by K.W. Phinney of the NIST Chemical Sciences Division.

Acquisition and preparation of the SRM were coordinated by K.E. Sharpless of the NIST Chemical Sciences Division.

Statistical analysis of the data was performed 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.

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Robert L. Watters, Jr., Director
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Analytical measurements were performed by J. Camara, E.L. Kilpatrick, and L.T. Sniegoski of the Chemical Sciences Division. Measurements at CDC were performed by M. Xu and reviewed by M.E. Rybak under the direction of C.M. Pfeiffer.

Certified Concentration Values: The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it incorporates Type B uncertainty components related to the analyses, and expresses both the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide and with its Supplement 1 [4–6]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte [4]. For the certified values shown below, $k = 2$. The measurand is the pyridoxal 5'-phosphate value listed in Table 1. Metrological traceability is to the SI derived unit for mass fraction (expressed as ng/g), mass concentration (expressed as ng/mL), and amount-of-substance concentration (expressed as nmol/L).

Table 1. Certified Concentration Values for Pyridoxal 5'-Phosphate

	Concentrations		
	(ng/g)	(ng/mL)[a]	(nmol/L)[b]
Level 1	4.49 ± 0.15	4.59 ± 0.16	18.6 ± 0.6
Level 2	8.81 ± 0.29	9.00 ± 0.29	36.4 ± 1.2

[a] Mass concentrations were calculated from mass fractions using the following measured serum densities: Level 1, 1.02213 g/mL and Level 2, 1.02138 g/mL. The uncertainty in the serum density measurements was incorporated in values that are reported relative to units of volume.
[b] Molar concentrations were calculated from mass concentrations using the relative molecular mass 247.14 g/mol.

Table 2. Information Concentration Values for 4-Pyridoxic Acid

	Concentrations		
	(ng/g)[a]	(ng/mL)[b]	(nmol/L)
Level 1	21.7	22.2	121
Level 2	36.3	37.1	202

[a] Mass fractions were calculated from mass concentrations using the measured serum densities: Level 1, 1.02213 g/mL and Level 2, 1.02138 g/mL.
[b] Mass concentrations were calculated from molar concentrations using the relative molecular mass 183.16 g/mol.

NOTICE AND WARNINGS TO USERS

SRM 3950 IS INTENDED FOR RESEARCH USE. THIS IS A HUMAN SOURCE MATERIAL. HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. The supplier of this serum has reported that each donor unit of serum or plasma used in the preparation of this product has been tested by a FDA-approved method and found non-reactive/negative for hepatitis B surface antigen (HbsAg), human immunodeficiency virus (HIV) 1 and 2 antibodies, and hepatitis C virus (HCV). However, no known test method can offer complete assurance that hepatitis B virus, HCV, HIV, or other infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 2 or higher as recommended for any POTENTIALLY INFECTIOUS HUMAN SERUM OR BLOOD SPECIMEN in the Centers for Disease Control/National Institutes of Health Manual [7].

INSTRUCTIONS FOR STORAGE AND USE

Storage: The serum is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. The material should be stored at or below –60 °C. This SRM should be handled under subdued lighting conditions (i.e., yellow light) [8,9].

Use: SRM 3950 is provided as frozen serum that should be allowed to thaw at room temperature (20 °C to 25 °C) under subdued light. After the material is thawed, it should be used immediately. The contents of the vial should then be gently mixed prior to removal of a test portion for analysis. Precautions should be taken to expose serum only under subdued lighting conditions.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: SRM 3950 was prepared by Aalto Scientific Ltd. (Carlsbad, CA). Level 1 is an unfortified pool of human serum. In order to achieve the desired level of PLP in Level 2, a human serum pool containing a naturally lower level was fortified with additional PLP.

Analytical Methods: NIST determined vitamin B_6 levels as PLP by ID LC-MS/MS [2]. A labeled internal standard (pyridoxal-[$^2H_3$] 5'-phosphate) was added to 0.5 g serum and allowed to equilibrate for 30 min. Serum proteins were precipitated by the addition of aqueous trichloroacetic acid followed by incubation at room temperature. After centrifugation, supernatants were analyzed by LC-MS/MS. The transitions at *m/z* 248.0 → *m/z* 149.9 and *m/z* 251.1 → *m/z* 153.0 were monitored for the unlabeled and labeled forms of the analyte, respectively. The CDC precipitated proteins with metaphosphoric acid, filtered the samples, and determined the vitamin B_6 vitamers pyridoxal 5'-phosphate and 4-pyridoxic acid by LC with chlorite post-column derivatization and fluorescence detection [3,10]. The B_6 vitamers were separated under isocratic conditions on a C_{18} column with a mobile phase comprised of aqueous phosphate buffer (with 0.2 % acetonitrile, volume fraction). The initial mobile phase was comprised of 100 % aqueous buffer, and a linear gradient from 0 % to 30 % methanol was employed after elution of the B_6 vitamers to facilitate column cleanup between injections.

Homogeneity Assessment: The homogeneity of PLP was assessed at NIST using the method and test portion size described above; analysis of variance 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.

[(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.

REFERENCES

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Certificate Revision History: 28 December 2015 (Change of expiration date; editorial changes); 20 July 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 http://www.nist.gov/srm.