National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 927d

Bovine Serum Albumin (7 % Solution)

(Total Protein Standard)

This Standard Reference Material (SRM) is intended primarily for use in the standardization of procedures employed in clinical analyses for total serum protein, for critical evaluation of daily working standards used in these procedures, and as a reference standard for assays of total protein by colorimetric methods. This SRM is a solution (mass fraction 7 %) of known protein concentration and purity. It conforms to the specification for standardized protein solution approved by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) [1]. The protein content of this SRM was determined using the biuret reference method [2] that is recommended for use in standardizing laboratory-prepared protein solutions and "normal" serum pools. Such standardized "normal" sera could then be used to calibrate refractometers or other instruments for serum protein estimations. SRM 927d may also be used for other procedures, such as gel diffusion, amino acid analysis, electrophoresis, nitrogen assays, or other tests that require well-characterized protein for calibration or evaluation. A unit of 927d consists of 10 ampoules, each containing 2.2 mL of solution.

In addition to the measurements using the biuret method, NIST made measurements of the bovine serum albumin (BSA) concentration using amino acid analysis. There is a discrepancy between the results from the two approaches. As the two different approaches are not determining the same measurand, it was decided to separately report the results from the two approaches as follows: 1) certified BSA concentration by amino acid analysis and 2) reference total protein concentration by the biuret method.

Certified Concentration Value: The certified concentration value for bovine serum albumin (BSA) as determined by amino acid analyses is provided in Table 1. Two different methods of amino acid analysis were used for value assignment (see "Analytical Methods" section). 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 [3].

Reference Values: The reference protein concentration determined using the biuret method is provided in Table 2. The biuret reference method [2] was employed to determine protein concentration in SRM 927d using SRM 927c as an external standard. Reference values are noncertified values that are the best estimates of the true values; however, the values 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 analytical methods [3]. Reference values are provided in Table 3 for additional properties including mean fill volume, pH, density, absorbances at various wavelengths, and relative molecular mass as determined using electrospray ionization mass spectrometry.

Expiration of Certification: The certification of **SRM 927d** is valid, within the measurement uncertainty specified, until **30 September 2016**, provided the SRM is handled in accordance with 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 Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before expiration, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The overall direction and coordination of technical measurements leading to the certification were performed by M.J. Welch of the NIST Chemical Sciences Division and D.M. Bunk of the NIST Biomolecular Measurement Division.

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Gaithersburg, MD 20899
Certificate Issue Date: 25 March 2014
Certificate Revision History on Last Page

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

Analyses were performed by D.M. Bunk, L.T. Sniegoski of the NIST Chemical Sciences Division, and M. Vergne formerly of the NIST Chemical Sciences Division.

Statistical analysis of the data used for certification was performed by N.F. Zhang of the NIST Statistical Engineering Division.

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

NOTICE AND WARNING TO USERS

SRM 927d IS INTENDED FOR IN-VITRO DIAGNOSTIC USE ONLY. The blood used in the preparation of SRM 927d Bovine Serum Albumin (7 % Solution) was collected from cattle sourced in the United States. Only blood from cattle/carcasses that have passed ante-mortem and post-mortem U.S. Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) inspection was used. This material was collected prior to any known case of Bovine Spongiform Encephalopathy (BSE) in the United States. There were no additives to the pooled serum prior to protein purification.

INSTRUCTIONS FOR STORAGE AND USE

Storage: This SRM is supplied to the user in sealed ampoules. The SRM should be stored in a refrigerator at a temperature between 2 °C and 8 °C. The ampoules should not be frozen because of possible breakage of ampoules during the thawing process. Once an ampoule is opened, the solution should be used promptly. Any unused solution in opened ampoules should be discarded.

Inappropriate Uses: This SRM is not intended to be used as a standard for dye-binding tests, for checking precalibrated refractometers, for immunochemical methods, or as an additive for bilirubin standardization.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Note: This material is intended for “in vitro” diagnostic use only. It was derived from whole bovine blood collected at a USDA licensed establishment. The supplier of this material has reported that this material was produced under sanitary conditions and was derived from clinically healthy animals.

Source of Material: The bovine serum albumin solution was prepared by Bionostics Inc., (Acton, MA, USA) under contract with Bioreclamation, Inc., (Hicksville, NY, USA). The bovine serum was produced for manufacture into products for pharmaceutical use by West Laboratories, Inc. at USDA EST. #245-J, Iowa Beef Packers, Inc., (Joslin, IL, USA).

The BSA for this SRM was dissolved in 0.02 mol/L sodium chloride and the pH adjusted to 6.5 to 6.8 with 1.0 mol/L sodium hydroxide. The material was sterilized by membrane filtration and tested for sterility by approved methods [4].

Preparation of Dilutions: Protein solutions of lower concentration may be prepared by transferring the appropriate aliquot to a volumetric flask and diluting to volume. Diluents are not furnished with the SRM; an aqueous sodium chloride diluent, such as a solution having a concentration of 0.15 mol/L, may be used.

Analytical Methods: All analyses for the certified and reference values were performed at NIST. For the determination of BSA, two different methods for determining amino acids were used. The first method involved isotope dilution liquid chromatography/tandem mass spectrometry (ID-LC/MS/MS). Samples of SRM 927d and SRM 927c (as a control) were hydrolyzed with vapor-phase hydrochloric acid (HCl) for 2 h at 150 ºC in sealed vials. After hydrolysis, the samples were lyophilized and then reconstituted with < 2 mL/L formic acid in acetonitrile containing isotope-labeled analogs of phenylalanine, proline, and valine. Amino acids were separated using hydrophilic interaction chromatography (HILIC) on a polyhydroxyethyl aspartate column with gradient elution. Measurements were performed on a triple quadrupole mass spectrometer, monitoring specific transitions for each amino acid. The measurements were calibrated with different dilutions of SRM 2389 Amino Acids in 0.1 mol/L HCl. Data were collected for phenylalanine, proline, and valine. Based upon the known amino acid sequence for BSA, the concentration of BSA was calculated from the concentrations determined for each of the

[(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.

amino acids. For the second method, a commercial amino acid analyzer was used following hydrolysis as described above. This analyzer uses an ion exchange resin column to separate the amino acids, followed by derivatization with ninhydrin, and absorbance detection at 570 nm and 440 nm. These measurements were calibrated with dilutions of SRM 2389. The amino acids used for quantitation were alanine, leucine, lysine, and aspartic acid/asparagine.

The reference total protein concentration was measured using the biuret reference method for total serum protein [2]. The measurements involve a direct comparison between the current SRM lot (927d) and the previous lot (927c) and were performed by spectrophotometry.

The reference values for various properties determined for SRM 927d are given in Table 3. Absorbances were measured in accordance with requirements specified for a standard BSA solution [1]. Measurement of the pH was performed using a pH meter with a glass body combination pH electrode calibrated with pH 4 and pH 7 buffers. Density was measured using the Lang-Levey pipet method [5]. Fill masses were determined gravimetrically, and fill volumes were calculated from the fill masses and mean density.

Relative molecular mass was determined using liquid chromatography/mass spectrometry (LC/MS). Measurements were performed on a single quadrupole mass spectrometer operated in the positive ion mode and coupled to a capillary LC with a commercial C4 column, 0.5 mm × 50 mm, held at 40 °C. Gradient elution using 0.05 mL/L trifluoroacetic acid in water and in acetonitrile was used. Horse apomyoglobin was used for mass calibration of the mass spectrometer. The molecular masses of the seven major forms of BSA found in SRM 927d are shown in Table 3 in decreasing order of abundance. The previous lot (SRM 927c) had a similar range of molecular masses.

Table 1. Certified Bovine Serum Albumin Concentration by Amino Acid Analysis[(a)]

BSA Concentration:	65.41 g/L ± 0.82 g/L

[(a)] The certified value is the equally weighted mean of results obtained from two chemically independent methods (see "Analytical Methods"). The expanded uncertainty in the certified concentration is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated based on a Bayesian approach in [6] and the ISO/JCGM Guide [7]. The coverage factor, $k = 2$, represents an approximate 95 % level of confidence.

Table 2. Reference Total Protein Concentration by the Biuret Method[(a)]

Protein Concentration:	70.10 g/L ± 0.74 g/L

[(a)] This method-specific result is a reference value. The expanded uncertainty in the reference concentration is calculated as $U = ku_c$. The uncertainty, u_c, is based upon quadratically combining the measurement uncertainty with the uncertainty in SRM 927c, which served as the external standard for the measurements. The major component in the uncertainty for SRM 927d is the uncertainty in the concentration of SRM 927c. The coverage factor, $k = 2$, represents an approximate 95 % confidence interval.

Table 3. Reference Values for Various Properties of SRM 927d[(a)]

Mean Fill Volume:		2.245 mL	±	0.006 mL
pH:		6.70	±	0.01
Density:		1.0178 g/mL	±	0.0003 g/mL
Spectral Properties				
Absorbance Ratio (A_{252}/A_{279}ratio @ 1.0 g/L)		0.467	±	0.002
		Absorbance		
Soret Band (Visible)	A_{405}	0.140	±	0.002
	A_{500}	0.0320	±	0.0021
	A_{600}	0.0187	±	0.0024

Major molecular forms of BSA in decreasing order of abundance

Relative Molecular Mass		
66 432	±	7
66 549	±	12
66 473	±	14
66 593	±	11
66 344	±	13
66 222	±	14
66 389	±	14

Theoretical BSA relative molecular mass from amino acid sequence [8]: 66 399

[(a)] The uncertainties in the reference values are calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated according to the NIST and ISO/JCGM Guides [7], where u_c is intended to represent the measurement error at the level of one standard deviation. The coverage factor, $k = 2$, represents an approximate 95 % confidence interval.

REFERENCES

[1] NCCLS Approved Standard ACS-1; *Specification for Standardized Protein Solution* (*Bovine Serum Albumin*); 2nd ed.; National Committee for Clinical Laboratory Standards: Villanova, PA (1979).

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[8] European Bioinformatics Institute; *UniProt Database*, Swiss Institute for Bioinformatics, and the Protein Information Resource; available at http://wwdw.uniprot.org (accessed Mar 2014).

Certificate Revision History: 25 March 2014 (Extension of certification period; editorial changes); **30 June 2010** (This revision includes an extension of the certification period and minor editorial changes.); **10 November 2009** (This revision reflects the deletion of an information value and editorial changes); **02 February 2006** (Original certification 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.