National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2925

Recombinant Human Serum Albumin Solution
(Primary Reference Calibrator for Urine Albumin)
(Frozen)

SRM 2925 Recombinant Human Serum Albumin (HSA) Solution is intended for use in calibration of liquid chromatography-tandem mass spectrometric procedures for the determination of albumin in urine. The concentration of recombinant HSA in this SRM was determined using amino acid analysis. A unit of SRM 2925 consists of two vials, each containing approximately 0.5 mL of solution. End users will need to evaluate the suitability of SRM 2925 for additional applications that require well-characterized protein for calibration or evaluation, including colorimetric assays for total protein, gel diffusion, amino acid analysis, electrophoresis, or proteomics-based experimental workflows.

Certified Concentration Value: The certified concentration value for recombinant HSA was determined through amino acid analysis using isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC-MS/MS) [1]. The measurand is the total concentration of recombinant HSA calculated using the amount-of-substance determined for each of the amino acids and the known amino acid sequence for HSA. Metrological traceability is to the SI derived units for molar concentration (expressed as nanomoles per gram).

Certified Recombinant HSA Concentration: 14.4 nmol/g ± 0.3 nmol/g

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 [2]. The certified concentration for recombinant HSA was determined using a higher-order reference measurement procedure [3] calibrated with amino acid certified reference materials. The uncertainty provided value is an expanded uncertainty about the mean to cover the measurand, consistent with the ISO/JCGM Guide [4]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined standard uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for this analyte [4]. For the certified value shown above, $k = 2$.

Expiration of Certification: The certification of **SRM 2925** is valid, within the measurement uncertainty specified, until **01 October 2024**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of Certification: NIST will monitor this SRM until its certification expires. If substantive technical changes occur that affect the certified values before this certificate expires, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of technical measurements leading to the certification were performed by A. Beasley-Green of the NIST Biomolecular Measurement Division. Additional technical guidance was provided by D.M. Bunk and K.W. Phinney of the NIST Biomolecular Measurement Division.

Acquisition of the material and certification measurements were performed by A. Beasley-Green.

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

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Gaithersburg, MD 20899
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Steven J. Choquette, Director
Office of Reference Materials

Non-Certified Values: The non-certified reference values for density and the concentration of recombinant HSA in SRM 2925 (expressed in terms of grams per liter) are listed in Table 1. Reference values are non-certified values that are 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 [2,4]. The reference value concentration of recombinant HSA in SRM 2925, expressed in terms of grams per liter, was calculated using the relative molecular mass (calculated from the amino acid sequence), the density value, and the certified recombinant HSA concentration value above. Metrological traceability is to the International System of Units (SI) measurement unit for concentration, expressed as grams per milliliter or grams per liter, as realized by the method used. The non-certified information value for relative molecular mass is also included in Table 1. An 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 [2]. Information values cannot be used to establish metrological traceability.

Table 1. Non-Certified Values for Properties of SRM 2925

Property	Reference Values
Density (20.0 °C)	1.00016 g/mL ± 0.00001 g/mL
Recombinant HSA Concentration in SRM 2925:	0.958 g/L ± 0.0220 g/L ($k = 2$)

Property	Information Value
Relative Molecular Mass (dimensionless):	66 394.56

NOTICE AND WARNING TO USER

SRM 2925 was expressed in *Pichia pastoris* yeast cells and has the potential to contain toxins that may pose a health risk. Normal caution and care should be exercised during the material's handling and use.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling and Storage: SRM 2925 is shipped frozen (on dry ice) and, upon receipt, should be stored frozen until ready for use. A freezer temperature of –20 °C is acceptable for storage for up to one week. If a longer storage time is anticipated, the material should be stored at or below –80 °C. The SRM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room (20 °C to 25 °C) or refrigerator (5 °C to 8 °C) temperatures may result in degradation or modification of the constituent protein.

Use: Frozen vials of the SRM to be analyzed should be removed from the freezer and allowed to stand on ice or at room temperature (20 °C to 25 °C) until thawed. After the material is thawed, it should be used immediately. The material should be mixed briefly with a vortex mixer before aliquots are withdrawn.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source of Material: SRM 2925 is a frozen aqueous solution of recombinant HSA, expressed in *P. pastoris*. The stock material was procured from Albumin Bioscience, a business unit of Albumin Therapeutics, LLC, (Huntsville, AL) in a stock buffer composed of 5 % solution of phosphate buffer saline containing 4 mmol/L sodium caprylate and 4 mmol/L acetyltryptophan. SRM 2925 was prepared at NIST by desalting the stock material via gel filtration chromatography (3,000 g/mol cutoff) and exchanging the stock buffer to 50 mmol/L ammonium bicarbonate in water.

Measurement of HSA Concentration by Amino Acid Analysis (ID-LC-MS/MS): All analyses for the certified and non-certified values were performed at NIST. The amino acid analysis method involved isotope dilution-liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) based on multiple reaction monitoring (MRM). For certification, randomly selected vials of SRM 2925 were combined with isotope-labeled analogs of alanine (Ala), phenylalanine (Phe), and arginine (Arg) and were hydrolyzed with vapor-phase hydrochloric acid (HCl) for approximately 24 h at 120 °C in sealed vessels. After hydrolysis, the samples were dried down and reconstituted with 0.1 mL/L hydrochloric acid (HCl) in water. The amino acids were separated using gradient-elution mixed-mode chromatography on a reversed-phase analytical column with embedded acidic ion-pairing groups. Measurements were performed on a triple quadrupole mass spectrometer, monitoring a specific transition for each amino acid. The measurements were calibrated using amino acid certified reference materials from the National Metrology Institute of Japan (NMIJ; Tsukuba, Japan). The molar concentration for the three amino acids (Ala, Phe, Arg) were converted to

[(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.

mass concentration (grams per liter) using the theoretical molar mass of mature, native HSA. The theoretical molar mass was calculated from the atomic masses of the total number of carbon (^{12}C), nitrogen (^{14}N), oxygen (^{16}O), sulfur (^{32}S), and hydrogen (^{1}H) elements in each amino acid for the mature (residues 25-609) and native (including 17 disulfide linkages) amino acid sequence of HSA (66 394.56 g/mol). The concentration of recombinant HSA in SRM 2925 was calculated as the mean of the concentrations determined for each of the three amino acids.

Additional Analyses: The non-certified density measurements were performed volumetrically using the pulsed excitation method on the DMA 5000M Density Meter at 20.0 ºC.

Homogeneity Analysis: Homogeneity was assessed prior to the certification analyses. A stratified sampling plan was devised to test for homogeneity across the lot of vials. There was no apparent trend in the data when plotted against the sequence in which the vials were filled.

Stability Analysis: Stability was assessed prior to the certification analyses. A random sampling scheme was used to examine the degree of total protein degradation associated with the potential temperature conditions encountered during shipment from NIST to the end-user. There was no apparent trend in the data, which suggests that routine shipping conditions will not affect the composition of the material over a one-month period.

Additional Resource: Full details on the production, analysis, and statistical evaluation of SRM 2925 are provided in: NIST Special Publication 260-199, *Certification of Standard Reference Material® 2925 Recombinant Human Serum Albumin Solution (Primary Reference Calibrator for Urine Albumin).* This publication is available free of charge on the SRM web site https://www-s.nist.gov/srmors/view_detail.cfm?srm=2925.

REFERENCES

[1] Bunk, D.M.; Lowenthal, M.S.; *Isotope Dilution Liquid Chromatography-Tandem Mass Spectrometry for Quantitative Amino Acid Analysis*; Methods Mol. Biol., Vol. 828, pp. 29–38 (2012).

[2] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/system/files/documents/srm/SP260-136.PDF (accessed Feb 2020).

[3] NCCLS; *Development of Definitive Methods for the National Reference System for the Clinical Laboratory, Approved Guideline*; NCCLS Publication NRSCL 1-A; National Committee for Clinical Laboratory Standards: Wayne, PA (1991).

[4] 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 (2008); available at https://www.bipm.org/en/committees/jc/jcgm/publications (accessed Mar 2022); 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 Mar 2022).

Certificate Revision History: 16 March 2022 (Update to intended use; editorial changes); 24 February 2020 (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; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.