National Institute of Standards & Technology

Certificate

Standard Reference Material® 927b

Bovine Serum Albumin (7 %* Solution)
(Total Protein Standard)

This Standard Reference Material (SRM) is intended primarily for use in calibrations, 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 certified as a solution of known protein concentration and purity. It conforms to the specification for standardized protein solution approved by the National Committee for Clinical Laboratory Standards (NCCLS) [1]. The Bovine Serum Albumin (7 %* solution) was brought to proper ionic strength with sodium chloride and to proper pH with sodium hydroxide. It was sterilized by membrane filtration and tested for sterility by approved methods [2]. A Biuret reference method [3], was employed to determine protein content in SRM 927b using SRM 927a as an external standard. It is suggested that this Biuret method be used to standardize 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 927b may also be used for other procedures, such as gel diffusion, amino acid analysis, electrophoresis, nitrogen assays, or other tests which require well-characterized protein for calibration or evaluation. A unit consists of 10 vials containing 2.1 mL each.

Certified concentration of peptide mass by a Biuret method [3] is: 72.01 g/L $\pm$ 0.33 g/L.

The uncertainty is computed according to the ISO Guide [4], and is at the 95 % level of confidence.

NOTICE AND WARNING TO USERS

This SRM is not to be used in standardizing dye-binding tests, for checking precalibrated refractometers, or as an immunochemical standard. It is not recommended for use in bilirubin standardization. This SRM is intended for "in vitro" diagnostic use only.

Source of Material: The bovine serum albumin solution was prepared by the Diagnostics Division, Miles Laboratories, Inc., Kankakee, IL.

The serum albumin material was derived from bovine blood collected at a USDA licensed establishment. The cattle received ante- and post-mortem health inspections at the abattoir by a U.S. Veterinary Services inspector and they were apparently free from infectious and contagious diseases. All donor animals were sourced from the United States, a country in which Bovine Spongiform Encephalopathy is not known to exist. Blood collection records are incorporated into the manufacturing batch documents of Miles Laboratories, Inc.

*The amount-of-substance fraction expressed in %.

The technical and support aspects involved in the update and revision of the certificate accompanying this SRM were coordinated through the Standard Reference Materials Program by J.C. Colbert.

Gaithersburg, MD 20899
June 6, 1995
(Revision of certificate dated 8-10-94)

Thomas E. Gills, Chief
Standard Reference Materials Program

Analyses were performed in the NIST Biotechnology and Analytical Chemistry Divisions by E. Grabbe, J.J. Edwards, Y.C. Wu, D. Feng, W.F. Koch, and T. Butler.

The statistical analysis of the data used for certification was performed by S.B. Schiller of the NIST Statistical Engineering Division.

The overall direction and coordination of technical measurements leading to the certification was performed in the NIST Biotechnology Division under the chairmanship of D.J. Reeder.

Stability: This SRM is supplied to the user in sealed ampules. Recommended storage temperature is refrigeration between 2 °C and 8 °C. The ampules should not be frozen because of possible breakage of ampules during the thawing process. Once an ampule is opened, the solution should be used promptly. Unused solution in opened ampules should be discarded. Under proper storage in sealed ampules, this SRM is expected to be stable for 3 years from date of shipment from NIST. Stock of this SRM kept at NIST will be monitored periodically and if degradation is evident, purchasers will be notified. Please return the attached registration form to facilitate notification.

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; however, an aqueous sodium chloride diluent, such as a solution having an amount-of-substance concentration of c (NaCl) = 0.15 mol/L, may be used.

REFERENCES

[1] Specification for Standardization Protein Solution (Bovine Serum Albumin), NCCLS Approved Standard: ASC-1, National Committee for Clinical Laboratory Standards, Villanova, PA, Second Edition, (1979).

[2] United States Pharmacopeia, 21st Revision, p.1350 Class A, United States Pharmacopeial Convention, Rockville, MD.

[3] Doumas, B.T., et. al., A Reference Method for the Determination of Total Protein, Clin. Chem. **27** (10): 1642-1654, (1981).

[4] *"Guide to the Expression of Uncertainty in Measurement"*, ISBN 92-67-10188-9 1st Ed. ISO, Switzerland, (1993).

[5] Reeder, D.J., Sniegoski, L.T., and Schaffer, R., *o*-Phthalaldehyde for the Fluorometric Assay of Nonprotein Amino Compounds, Anal. Biochem **86**: 490-497, (1978).

Information Values: The following values are not certified, but provided for information only. The uncertainties where given, were computed according to the ISO Guide [4], and at the 95 % level of confidence.

Minimum Fill Volume	2.175 mL	
pH	6.662	± 0.0122
Sodium by Flame Emission Spectroscopy	28.43 mmol/L	± 0.42 mmol/L
Chloride by Ion Chromatography	0.0194 mol/L	± 0.0005 mol/L
Density	1.021 g/mL	± 0.007 g/mL

Spectral Properties	Absorbance
Ultraviolet (A_{252}/A_{279}ratio @ 1.0 g/L)	0.474 ± 0.016
Soret Band (A_{405} @ 72 g/L)	0.1543 ± 0.0027
Visible (A_{500} @ 72 g/L)	0.0407 ± 0.0022
(A_{600} @ 72 g/L)	0.0218 ± 0.0023
Extinction Coefficient @ 278 nm for 0.1 g/100 mL	0.665

Constituent	Mass Fraction Found
Protein/Dimer by HPLC	0.017 ± 0.001
Non-protein Amino Compounds by Fluorometric Assay [a], [5]	0.005 ± 0.002

[a]The uncertainty is based on a 95 % confidence, 95 % coverage statistical tolerance interval with an additional allowance for a trend in the measurement process.

The protein composition of SRM 927b was calculated as a percentage of total protein. Quantitative estimates were determined by 2-dimensional electrophoresis, silver staining, and computer imaging. Six (6) samples were analyzed.

Albumin	(% Total) Avg: 99.36 Range: 98.95 to 99.93	(% Monomer) Avg: 83.48 Range: 74.61 to 90.74
	(% Dimer) Avg: 7.82 Range: 4.42 to 12.57	(% Proteolytic Products) Avg: 8.06 Range: 4.00 to 14.16
Contaminants	(% Total) Avg: 0.64 Range: 0.06 to 1.05	