

National Bureau of Standards Certificate

Standard Reference Material 926

Bovine Serum Albumin (Total Protein Standard)

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This Standard Reference Material (SRM) is certified as a chemical of known purity. It conforms to the specifications for bovine serum albumin (lyophilized powder) approved by the National Committee for Clinical Laboratory Standards [1]. A 7-percent solution of this albumin is issued as a standardized solution (SRM 927) for use in the calibration and standardization of procedures for total serum protein determinations and for routine critical evaluation of daily working standards used in these procedures.

Table 1. Certified Data

<u>Constituent</u>	<u>Mass Fraction Found</u>
Ash	0.0006 ± 0.0001
Carbohydrate	
Total hexose	0.00034 ± 0.00003
Sum of citrate, pyruvate, lactate	0.00015 ± 0.00002
Lipid (Total)	0.0003 ± 0.0003
Non-protein amino compound, as norleucine	0.00002 ± 0.00002
Water	
Karl Fischer method	0.048 ± 0.002
Vacuum oven method	0.0475 ± 0.0006
Protein impurities (Dimer)	
Cellulose acetate	None detected
Gel electrophoresis (polyacrylamide gel)	0.043 ± 0.002
Gel chromatography (Sephadex)	0.050 ± 0.003
<u>Spectral properties</u>	<u>Absorbance</u>
Ultraviolet, (A ₂₅₂ /A ₂₇₉ ratio)	0.485 ± 0.009
Soret band, (A ₄₀₅ at 70 g/L)	0.182 ± 0.002
Visual, (A ₅₀₀ at 70 g/L)	0.048 ± 0.002
(A ₆₀₀ at 70 g/L)	0.023 ± 0.002

The uncertainty is expressed as one standard deviation. The spelling "liter" and its symbol "L" are recommended usage in the United States (See Federal Register, Vol. 41, 54018, Dec. 10, 1976).

The bovine serum albumin used for this Standard Reference Material was prepared by the Research Division, Miles Laboratories, Inc., Kankakee, Illinois. Analyses were performed by R. G. Christensen, A. Cohen, R. Deardorff, R. Durst, H. Hertz, B. Howell, S. Margolis, J. Maurey, D. J. Reeder, L. Sniegowski, P. Verdier, H. L. Wagner, F. W. Wang, and W. Yap.

The overall direction and coordination of technical measurements leading to the certification were under the chairmanship of R. Schaffer.

The technical and support aspects concerning the preparation, certification, and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

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J. Paul Cali, Chief
 Office of Standard Reference Materials

(over)

The analytical techniques used in the certification of this Standard Reference Material are generally those recommended in NCCLS Approved Standard: ACC-1 [1]. Non-protein amino compounds were determined by fluorescence methodology [2].

Supplementary information for this Standard Reference Material is given in Table 2; the amino acid composition of the Bovine Serum Albumin in Table 3; and the fatty acid composition of the included lipid in Table 4. These data are not certified, but given for information only.

Table 2 — Supplementary information on SRM 926, Bovine Serum Albumin

Refractive index increment (dn/dc) (cm ³ /g)	0.18440 ±0.00025
Partial specific volume (in vacuo) (cm ³ /g)	0.7332 ±0.0007
Nitrogen analysis (Kjeldahl) (mg/g)	163.98 ±1.15
Immunoelectrophoresis	No protein impurities detected
Molecular weight (g/mol) ^a	68,400 ±110
Number-average molecular weight of 7-percent solution, M _n /C ₀ ^b	9.73x10 ⁵ ±0.11x10 ⁵

^aWeight-average molecular weight, M_w, determined by equilibrium centrifugation.

^bDetermined by membrane osmometry, where C₀ is concentration of albumin standard (SRM 927).

Table 3 — Amino acid composition of SRM 926, Bovine Serum Albumin

<u>Amino Acid</u>	<u>Residues per 100 Residues</u>
Aspartic acid	9.13
Threonine	5.49
Serine	4.61
Glutamic acid	13.89
Proline	5.73
Glycine	2.72
Alanine	7.98
1/2-Cystine	5.51
Valine	6.30
Methionine	0.67
Isoleucine	1.97
Leucine	11.03
Tyrosine	3.36
Phenylalanine	4.86
Lysine	9.98
Histidine	2.85
Arginine	3.93
Tryptophan	Not determined

Table 4 — Fatty acid composition by gas chromatography-mass spectrometry of the extracted lipid.

<u>Compound</u>	<u>Relative Amount</u>
Methyl benzoate	4.4
Methyl decanoate	3.0
Methyl dodecanoate	3.9
Methyl tetradecanoate (myristate)	10
Methyl pentadecanoate	6.9
A phthalate ester	7.1
Methyl hexadecanoate ^(a)	20
Methyl hexadecanoate (palmitate)	45
Dibutyl phthalate	100
Methyl oleate	12
Methyl octadecanoate (stearate)	23
Methyl eicosanoate	8.1

a = probable identity