National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6202-a
No. +++

Human Serum Albumin

This certified reference material (CRM) is produced in accordance with the NMIJ's management system and is in compliance with ISO 17034 and ISO/IEC 17025. It is an aqueous solution of human serum albumin. This CRM is primarily intended for use in the calibration and validation of analytical methods and instruments for the determination of albumin by instrumental analyses such as amino acid analysis, chromatography, and spectrophotometry. It is also intended for use in controlling the precision of said analytical methods and instruments.

Certified Value

The certified value, expressed as mass concentration of albumin at 20 °C, is given in the table below. The uncertainty of the certified value is the half-width of the expanded uncertainty interval calculated using a coverage factor (k) of 2, which gives a level of confidence of approximately 95 %.

	CAS No.	Certified value Mass concentration (g/L)	Expanded uncertainty Mass concentration (g/L)
Albumin	70024-90-7	74.3	2.1

Analytical Methods

The sample was hydrolyzed by hydrochloric acid, and the resulting hydrolysate was employed in amino acid analyses by isotope-dilution mass spectrometry. The results of the amino acid analyses were converted into the mass concentration by the density and molecular weight to give the certified value.

Amino acid analyses were conducted by the following two different methods:

(1) Microwave-assisted liquid-phase hydrolysis, followed by reversed-phase chromatography/tandem mass spectrometry utilizing the pre-column derivatization method:

The hydrolysis was performed by microwave-assisted liquid-phase hydrolysis with hydrochloric acid at 160 °C for 3 h. The hydrolyzed amino acids—aspartic acid, glutamic acid, proline, lysine, valine, isoleucine, leucine, and phenylalanine—were quantified using *N*-butylnicotinic acid *N*-hydroxysuccinimide ester iodide as the derivatization reagent.

(2) Gas-phase hydrolysis, followed by reversed-phase chromatography/tandem mass spectrometry utilizing the pre-column derivatization method:

Gas-phase hydrolysis was performed with hydrochloric acid at 130 °C for 24 h. The hydrolyzed amino acids were modified with 1-bromobutane as the derivatization reagent and quantified as in (1).

Metrological Traceability

The certified value is traceable to the International System of Units (SI) via amino acid analysis based on the primary method, isotope-dilution mass spectrometry calibrated with amino acid CRMs: L-aspartic acid (NMIJ CRM 6027-a), L-glutamic acid (NMIJ CRM 6026-a), L-proline (NMIJ CRM 6016-a), L-lysine monohydrochloride (NMIJ CRM 6018-a), L-valine (NMIJ CRM 6015-a), L-isoleucine (NMIJ CRM 6013-a), L-leucine (NMIJ CRM 6012-a), and L-phenylalanine (NMIJ CRM 6014-a).

Indicative Value

The indicative value is given in the table below, which is the concentration of albumin determined by the biuret method[1)] using bovine serum albumin CRM, SRM 927d, from the National Institute of Standards and Technology (NIST), as the standard solution. The indicative value can be used for the determination of proteins by the biuret method when traceability to NIST SRM 927d is required. The uncertainty of the indicative value was obtained by combining uncertainties originating from value-assignment, homogeneity, stability, and the standard solution. The uncertainty of the indicative value is the half-width of the expanded uncertainty interval calculated using a coverage factor (k) of 2, which gives a level of confidence of approximately 95 %.

[1)]B. T. Doumas., D. D. Bayse, R. J. Carter, T. Peters Jr, R. Schaffer. A candidate reference method for determination of total protein in serum. I. Development and validation. Clin. Chem. (1981) 27, 1642-50.

	Indicative value Mass concentration (g/L)	Expanded uncertainty Mass concentration (g/L)
Albumin	77.3	3.2

Expiration of Certification

The certificate is valid for one year from the date of shipment, provided that the material is stored in accordance with the instructions given in this certificate.

Sample Form

This CRM is in the form of a clear and colorless liquid. This CRM of ca. 1 mL in net volume is kept in a glass ampule, and the ampule is sealed in a plastic bag and encapsulated with an ampule-cutter in a polystyrene tube.

Homogeneity

The homogeneity of this CRM was verified by measuring albumin by liquid chromatography in 10 ampules taken by stratified random sampling from among 430 samples of the whole batch. The measurements were performed with reversed-phase chromatography using 0.1 g of this CRM. The homogeneity is reflected in the uncertainty of the certified value.

Instructions for Storage

This CRM should be stored under refrigeration (about 4 °C) without allowing it frozen.

Instructions for Use

This CRM is for laboratory use only and not for *in vivo* use. The material should be mixed thoroughly by gentle inversion before use. This CRM should be used promptly once the ampule is opened. Take care to prevent absorption onto labware when the material is diluted. The commutability of the material for each particular assay is not confirmed.

NOTICE AND WARNINGS TO USERS

This CRM was made from pooled human serum. HBs antigen, HCV antibody, HIV-1/HIV-2 antibodies, and gene screening for these viruses were found to be negative. However, HANDLE THE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE. THIS CRM MUST BE HANDLED WITH ADEQUATE CARE AS A PATIENT SPECIMEN, SUCH AS USING SAFETY GLOVES AND STERILIZING CONTAMINATED LABWARE. Refer to the safety data sheet (SDS) on this CRM before use.

Preparation

This CRM was prepared from commercial highly purified human serum albumin (Sigma-Aldrich Co., fatty acid-free and globulin-free grade) produced from pooled human serum. Preparation of the aqueous solution and filling of the glass ampules were performed by Wako Pure Chemical Industries, Ltd.

Technical Information

(1) The solution composition of this CRM is 20 mmol/L NaCl, 0.05 % NaN_3 (pH 6.7).

(2) The densities of this CRM are 1.0201 g/cm^3 at 20 °C and 1.0190 g/cm^3 at 25 °C.

(3) The molecular weight of human serum albumin used here is 66 402. This molecular weight was calculated based on the 25–609 amino acids of the human albumin sequence (UniProt, accession # P02768) considering 17 of the disulfide bonds as the secreted form. The measured relative molecular mass of this CRM by mass spectrometry showed a value close to the above molecular weight.

(4) The concentration of this CRM without considering the molecular weight and density is (1098 ± 31) µmol/kg. [The number following ± represents the expanded uncertainty with a coverage factor (k) of 2.]

(5) This CRM contains dimeric and trimeric forms of albumin, in addition to the monomer. The relative area percentages of UV absorption by gel filtration chromatography were as follows at the time of certification. (The numbers following ± represent the deviations of measurement.) These values may represent the percentages in the mass.

Monomer: (89.6 ± 0.02) %
Dimer: (9.42 ± 0.02) %
Trimer: (1.00 ± 0.01) %

The percentage of monomers increased and the percentages of dimers and trimers decreased upon storage at 4 °C. When the test samples were used, the percentage of monomeric form increased approximately 3 % in one year.

NMIJ Analysts

The technical manager for this CRM is TAKATSU A. and the production manager is KINUMI T. The analysts are KINUMI T., SAKAGUCHI Y. and MIZUNO R.

Information

If substantive technical changes occur that affect the certification before the expiration of this certificate, NMIJ will notify the registered customer. Customer registration on the NMIJ Website (given below) will facilitate notification. Technical reports regarding this CRM can be obtained from the contact details given below.

Reproduction of Certificate

In reproducing this certificate, it should be clearly indicated that the document is a copy.

April 1, 2020

ISHIMURA Kazuhiko
President
National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact:
National Institute of Advanced Industrial Science and Technology,
National Metrology Institute of Japan (NMIJ),
Center for Quality Management of Metrology, Reference Materials Office,
1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan
Phone: +81-29-861-4059; Fax: +81-29-861-4009; https://unit.aist.go.jp/nmij/english/refmate/

Revision history
April 1, 2015: “Metrology Management Center” was renamed to “Center for Quality Management of Metrology.”