Chemical Metrology Division
Applied Sciences Group
Health Sciences Authority
1 Science Park Road, #01-05/06,
The Capricorn, Singapore Science Park II,
Singapore 117528
Tel: 65 6775 1605 Fax: 65 6775 1398
Website: www.hsa.gov.sg
Email: HSA_CML@hsa.gov.sg

Ref. No.: CML-HRM-3009A/03
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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-3009A

Human Serum Albumin Solution

Batch Number
STY-0127-002

Foreword
A unit of the certified reference material (CRM) HRM-3009A consists of one vial of recombinant human serum albumin in buffer solution (20 mM sodium chloride, 0.05 % sodium azide, pH 6.7). Each vial contains 500 µL of solution. The material appears as a clear liquid after thawing.

The CRM was produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO 33405:2024 [3].

Certified Concentration and Mass Fraction Values
The certified concentration and mass fraction values for human serum albumin in HRM-3009A are given in Table 1. The certified mass fraction in g/kg was calculated from the concentration in mol/kg and the relative molecular mass of human serum albumin (66437 g/mol) calculated using the amino acid sequence of human serum albumin. The certified concentration in g/L was calculated from the mass fraction in g/kg and the measured solution density at about 22 °C (1.00133 ± 0.00033 g/mL).

Table 1. Certified Concentration and Mass Fraction Values of Human Serum Albumin Solution

Analyte	Concentration in mmol/kg	Mass Fraction in g/kg	Concentration in g/L
Human Serum Albumin	**0.1506 ± 0.0036**	**10.01 ± 0.24**	**10.02 ± 0.24**

Each certified value is expressed as the certified value ± the expanded uncertainty.

The certified value is the mean of measurements of six samples taken from a minimum of three vials. The certified mass fraction value for HRM-3009A was determined using isotope dilution mass spectrometry (IDMS). A four-point calibration curve was used in the measurements.

The associated measurement uncertainty of the certified value was evaluated in accordance with

ISO/IEC Guide 98-3:2008 [4]. The expanded uncertainty (coverage factor of 2) corresponded to a level of confidence of about 95%.

Validity

The certified values of HRM-3009A are valid within the specified measurement uncertainty until **14 April 2026**. The validity of HRM-3009A will be extended if it is tested to be sufficiently stable for continuous use. The certified values of HRM-3009A are invalid when the solution has deteriorated or is mishandled.

Source of Materials

The albumin solution was prepared by dissolving lyophilised albumin from a commercial source in a buffer solution (20 mM sodium chloride, 0.05 % sodium azide, pH 6.7).

Homogeneity

Homogeneity testing was performed on two subsamples taken from eleven vials using a high performance liquid chromatography-diode array detector (HPLC-DAD) system. The sample size taken for homogeneity testing was 2 µL. No significant differences in the between and within-vial variances were found using *F*-test (ANOVA) at 95 % confidence level [3]. The u_{bb} was evaluated from the uncertainty due to between-vial inhomogeneity.

Stability

Short-term stability of albumin in HRM-3009A was conducted over a period up to 14 days at −20 °C, up to 7 days at 4 °C, and up to 2 days at 25 °C using HPLC-DAD. The results showed that the CRM was stable over the study period at the three temperatures.

Long term stability of albumin in HRM-3009A stored at a temperature of below −60 °C was evaluated on three occasions over a period of up to seven months using a liquid chromatography-isotope dilution tandem mass spectrometric (LC-IDMS/MS) method. The results showed that the CRM was stable when stored at below −60 °C over the study period [3]. The u_{stab} was evaluated from the standard error of the slope.

Analytical Methods

For determination of the concentration of albumin, a fully validated LC-IDMS/MS method modified from a previously published protocol was used [5]. Five amino acids calibration standards (leucine, isoleucine, phenylalanine, proline, and valine) were used for quantification. The method involved spiking with $^{13}C_6$-L-leucine, $^{13}C_6$,^{15}N-L-Isoleucine, $^{13}C_6$-L-Phenylalanine, $^{13}C_5$,^{15}N-L-Proline, and $^{13}C_5$-L-Valine, followed by hydrolysis of albumin using 8 M hydrochloric acid. Chromatographic separation of the resulting L-proline, L-leucine, L-isoleucine, L-phenylalanine and L-valine was achieved using an Agilent Zorbax Eclipse AAA (4.6 x 75 mm, 3.5 µm) column, followed by tandem mass spectrometric measurement of the five amino acids.

Metrological Traceability

The certified values are traceable to the International System of Units (SI) established through the use of L-proline CRM (HRM-1007A), L-leucine CRM (HRM-1008A), L-isoleucine CRM (HRM-1013A), L-phenylalanine CRM (HRM-1014A) and L-valine CRM (HRM-1006A) from Health Sciences Authority.

Intended Use

HRM-3009A is a primary measurement standard and is intended for use as a calibrant for the determination of albumin in human urine. Users may refer to ISO 33403:2024 [6] for the recommended statistical treatment of the certified reference value and the associated uncertainty of the CRM as control materials.

Warning and Safety Precautions for Users

Treat the material as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation.

Instructions for Use

Prior to use, the CRM should be thawed at room temperature (between 18 °C to 25 °C) and mixed well by gentle vortexing. After use, the vial should be tightly capped immediately and stored at below −60 °C. The certified values remain valid for up to four freeze-thaw cycles, as the stability of the CRM subjected to such conditions has been investigated. If results differ from certified value in subsequent sampling, customers are advised to purchase a new CRM.

The recommended minimum sample size for each use is 2 µL. The certified value may not be valid if smaller amounts are taken.

Transport and Storage

The CRM is transported in frozen state (in dry ice). Upon receipt, it should be stored at below −60 °C. The CRM should not be exposed to sunlight or ultraviolet radiation. Storage of the thawed material at room temperature or in the refrigerator may result in changes in the certified values.

Further Information

Please direct all enquiries regarding this CRM to the contact in this Certificate.

References

[1] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
[2] ISO 17034:2016 General requirements for the competence of reference material producers.
[3] ISO 33405:2024 Reference materials – Approaches for characterization and assessment of homogeneity and stability.
[4] ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995).
[5] Hong Liu, Lingkai Wong, Sharon Yong, Qinde Liu, Tong Kooi Lee. Anal Bioanal Chem, 2015, 407, 7579-7587.
[6] ISO 33403:2024 Reference materials – Requirements and recommendations for use.

Certificate Revision Records

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-3009A/01	14 Apr 2025	Issuance of first certificate
CML-HRM-3009A/02	09 Oct 2025	Revision of intended use
CML-HRM-3009A/03	27 Oct 2025	Revision of units of certified values

Note
HSA does not assume any liability with respect to any loss caused by improper use and/or storage of the CRM by the customer.

Dr Teo Tang Lin
Division Director
Chemical Metrology Laboratory
Chemical Metrology Division