

Certificate of Analysis

CERTIFIED REFERENCE MATERIAL HRM-3014A

Human Serum Albumin Solution for Total Protein Measurement

Batch Number

STY-0207-001

Foreword

A unit of the certified reference material (CRM) HRM-3014A 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 1 mL 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 Value

The certified concentration for human serum albumin in HRM-3014A is given in Table 1.

Table 1. Certified Concentration Value of Human Serum Albumin Solution

Analyte	Certified Concentration (g/L)
Human Serum Albumin	63.3 ± 1.2

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

The certified value is the mean of measurements of twelve samples taken from three vials. The certified concentration value for HRM-3014A was determined using UV-Vis spectrophotometry method.

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 value of HRM-3014A is valid within the specified measurement uncertainty until **10 July 2027**. The validity of HRM-3014A will be extended if it is tested to be sufficiently stable for continuous use. The certified value of HRM-3014A is 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-3014A was conducted over a period up to 14 days at –20 °C and up to 7 days at 4 °C using a HPLC-DAD method. The results showed that the CRM was stable over the study period at the two temperatures.

Long term stability of albumin in HRM-3014A stored at a temperature of below –60 °C was evaluated on at least three occasions over a period of up to six months using a liquid chromatography-isotope dilution tandem mass spectrometric (LC-IDMS/MS) method and eight months using a HPLC-DAD 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

The concentration of albumin was determined using a fully validated biuret method with a spectrophotometer coupled with a fiber optic probe [5].

Metrological Traceability

The certified value is traceable to the bovine serum albumin CRM (SRM 927d) from the National Institute of Standards and Technology (NIST) through the use of human serum albumin CRM (NMIJ CRM 6202-a) from the National Metrology Institute of Japan as the calibration standard.

Intended Use

HRM-3014A is a primary measurement standard and is intended for use as a calibrant for the determination of total protein in different matrices. 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.

The recommended minimum sample size for each use is 2 µL. The certified value may not be valid if smaller amounts are taken. The certified value may not be valid for re-thawed and opened vials as the stability of CRM subjected to such conditions has not been investigated.

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 value.

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] Sharon Yong, Cheng Yang Ng, Hong Liu, Yiting Chen, Qinde Liu, Tang Lin Teo, et al. Anal Bioanal Chem, 2024, 416(28), 6611-6620.
- [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-3014A/01	10 Jul 2026	Issuance of first certificate

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.



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