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Ref. No.: CML-HRM-3010A/03
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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM-3010A

Glycated VHLTPE Peptide Solution

Batch Number
STY-0182-001

Foreword
A unit of the certified reference material (CRM) HRM-3010A consists of one vial of glycated VHLTPE (GE) peptide [1] in 20 mM ammonium acetate/acetonitrile (90:10, v/v) solution. The vial contains 1 mL of peptide solution. HRM-3010A is used in conjunction with HRM-3011A (VHLTPE Peptide Solution) as the calibrant for the determination of the amount-of-substance fraction of haemoglobin A1c (HbA1c).

The CRM was produced with reference to the requirements set out in ISO/IEC 17025:2017 [2], ISO 17034:2016 [3] and ISO 33405:2024 [4].

Certified Mass Fraction Value
The certified mass fraction value for GE in HRM-3010A is given below.

Certified Mass Fraction Value: 231.4 ± 5.6 mg/kg

The certified value is the mean of measurements of six samples taken from a minimum of three bottles. The certified mass fraction value for HRM-3010A was determined using isotope dilution mass spectrometry (IDMS). A four-point calibration curve was used in the measurements.

The associated measurement uncertainty of each certified value was evaluated in accordance with ISO/IEC Guide 98-3:2008 [5]. The expanded uncertainty (coverage factor of 2) corresponded to a level of confidence of about 95%.

Validity
The certified value of HRM-3010A is valid within the specified measurement uncertainty until **01 November 2027**. The validity of HRM-3010A will be extended if it is tested to be sufficiently stable for continuous use. The certified value of HRM-3010A is invalid when the solution has deteriorated or is mishandled.

Source of Materials

The GE solution was prepared by purifying a synthetic GE peptide material from GL Biochem (Shanghai) Ltd. using high performance liquid chromatography-diode array detector (HPLC-DAD) with a fraction collector system.

Homogeneity

Homogeneity testing was performed on two subsamples taken from eleven vials using a HPLC-DAD system. The sample size taken for homogeneity testing was 10 µL. No significant differences in the between and within-vial variances were found using *F*-test (ANOVA) at 95 % confidence level [4]. The u_{bb} was evaluated from the uncertainty due to between-vial inhomogeneity.

Stability

The short-term stability testing was conducted on three occasions over a period up to 7 days at 4 °C and up to 2 days at 25 °C using a liquid chromatography-tandem mass spectrometric (LC-MS/MS) method with D_7-GE as internal standard. The results showed that the CRM was stable over the study period at both temperatures.

The long-term stability of the CRM stored at a temperature of -20 °C was evaluated on three occasions over a period of up to five months using a liquid chromatography-isotope dilution tandem mass spectrometric (LC-IDMS/MS) method. The results showed that the CRM was stable over the study period [4]. The u_{stab} was evaluated from the standard error of the slope.

Analytical Methods

For determination of the mass fraction of GE, a fully validated LC-IDMS/MS method was used [6]. The method involved spiking with $^{13}C_5$,^{15}N-L-proline and $^{13}C_6$-L-leucine, hydrolysis of peptide using 6 mol/L hydrochloric acid, separation of the resulting L-proline and L-leucine using an Agilent Zorbax Eclipse AAA (4.6 x 75 mm, 3.5 µm) column, followed by tandem mass spectrometric measurement of the L-proline and L-leucine.

Metrological Traceability

The certified mass fraction value is traceable to the International System of Units (SI) through the use of L-proline CRM (HRM-1007A) and L-leucine CRM (HRM-1008A) from Health Sciences Authority.

Intended Use

HRM-3010A is a primary measurement standard and is intended for use as a calibrant for the determination of HbA1c in human blood, which is used in the determination of the amount-of-substance fraction of HbA1c. Users may refer to ISO Guide 33:2015 [7] 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 ºC to 25 ºC) and mixed well by gentle vortex. After use, the CRM should be tightly capped immediately. The certified value remains valid when the CRM is re-thawed and opened for up to two times, as the stability of the CRM subjected to such conditions has been investigated.

The recommended minimum sample size for each use is 10 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 – 20 ^{o}C. The CRM should not be exposed to sunlight or ultraviolet radiation.

Further Information

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

References

[1] IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN), Nomenclature and symbolism for amino acids and peptides. Recommendations 1983.

[2] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.

[3] ISO 17034:2016 General requirements for the competence of reference material producers.

[4] ISO 33405:2024 Reference materials – Approaches for characterization and assessment of homogeneity and stability.

[5] ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995).

[6] Hong Liu, Lingkai Wong, Sharon Yong, Qinde Liu, Tong Kooi Lee. Analytical Bioanalytical Chemistry, Vol 407, 2015, p 7579-7587.

[7] ISO Guide 33:2015 Reference materials – Good practice in using reference materials.

Certificate Revision Records

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-3010A/01	01 Nov 2023	Issuance of first certificate
CML-HRM-3010A/02	28 Oct 2024	Extension of expiry date
CML-HRM-3010A/03	21 Oct 2025	Extension of expiry date

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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