TÜBİTAK
ULUSAL METROLOJİ ENSTİTÜSÜ

Certificate of the Reference Material

Name of the Material	:	25-hydroxy vitamin D_2 and 25-hydroxy vitamin D_3 in lyophilized serum
Material Code	:	UME CRM 1308
Issue Date	:	09.01.2015
Revision Date	:	18.09.2019 (Revision history can be found on the last page)
Validity Period of the Certificate	:	3 years from the sales date
Certified Values	:	

Measurand	Certified Value [1]	Uncertainty [2]	Certified Value [1,3]	Uncertainty [2,3]
25-hydroxy vitamin D_2 concentration in lyophilized serum	50.0 ng/g	2.9 ng/g	51.0 ng/mL	3.0 ng/mL
25-hydroxy vitamin D_3 concentration in lyophilized serum	48.8 ng/g	2.6 ng/g	49.8 ng/mL	2.7 ng/mL

[1] Certified values are the mean of 6 measurement results obtained from two units of the CRM on two different days by ID-LC-MS technique. The certified values and the uncertainties are traceable to the International System of Units (SI).

[2] The expanded uncertainty of certified value includes characterization, homogeneity, stability components and is stated as the standard uncertainty of measurement multiplied by the coverage factor k = 2, which for a normal distribution corresponds to a coverage probability of approximately 95%. The standard uncertainty of measurement has been determined in accordance with GUM "Guide to the Expression of Uncertainty in Measurement".

[3] Certified values and the uncertainties are calculated from the mass fraction (ng/g) using density of the material (1.0206 g/mL) measured at 22 °C.

TÜBİTAK UME, as a reference material producer, has been accredited by TÜRKAK according to TS EN ISO 17034 with the accreditation number AB-0001-RM.

Sales Date

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Director

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Informative Values

Parameter	Value
Density (22 °C)	1.0206 g/mL[1]

[1] Density of the reconstituted material was measured at 22 °C using a calibrated pipette and balance.

Additional measurement results of the interlaboratory comparison study, ID-LC-MS/MS and HPLC-UV techniques are presented in the UME CRM 1308 certification report.

Description

The Certified Reference Material is a solid material in an amber glass bottle with screw cap obtained by lyophilization of horse serum. Additional information is given in the certification report.

Intended Use

This material is intended to be used for method performance check and validation purposes of 25-hydroxy vitamin D_2 and 25-hydroxy vitamin D_3 in serum with LC-MS, LC-MS/MS and HPLC-UV methods.

UME CRM 1308 is suitable for determination of 25-hydroxy-vitamin D_2 and 25-hydroxy vitamin D_3 concentrations in human serum using LC-MS, LC-MS/MS and HPLC-UV methods.

UME CRM 1308 is not suitable for the calibration of 25-hydroxy-vitamin D_2 and 25-hydroxy vitamin D_3 measurements in human serum using immunoassay methods unless the commutability of the material is proven by the user.

Assessment of method performance can be done by comparing the measurement results with the certified values. The procedure for comparison is presented in the certification report.

Instructions for Use

All content of the unit should be reconstituted at once according to the recommended protocol presented below:

- UME CRM 1308 and the deionized water should be kept at room temperature at least for one hour in the room with balance before weighing.
- Before opening, the bottle should be tapped gently to table or bench top in order to collect all of the content at the bottom of the bottle.
- Screw cap is opened. The bottle with the inner cap is placed to the balance and tare is pressed. Unit is opened at vertical position gently by balancing the inner pressure with outer

pressure. Care should be taken in order not to lose any sample on cap or elsewhere. 2 mL of water is then added gently with a calibrated pipette into the sample. Then the inner cap is closed.

- Bottle, with its content and inner cap is weighed (m).
- The average amount of water added to the analysed units at TÜBİTAK UME was

$$m_{avg} = (1.9787 \pm 0.0200)\ \text{g}\ (k = 2)$$

If m is deviating from m_{avg}, then the corrected value of the analyte concentrations can be calculated as:

$$\text{Corrected analyte concentration} = \text{Certified value} \times \frac{m_{avg}}{m}$$

The bottle with water should be gently shaken with its cap and inner cap closed. If material is stacked to the inner cap, user can gently rotate the bottle upside down several times to dissolve those particles. Solid particles should be inspected and dissolving procedure should be repeated until all of the content is dissolved. User should perform the measurement on the dissolved material as quickly as possible. If it is necessary to keep the dissolved material for later measurements, it should be portioned into the small volumes and stored at temperatures equal to or lower than -20 °C in dark conditions. Subsamples should be stored in the bottles resistive to evaporation and sun light.

The minimum sample intake suggested for the reconstituted sample is 400 µL. The material can be safely dispatched at ambient temperature where the temperature does not exceed 25 °C and the transportation period of 4 weeks.

Storage Conditions

The material should be stored at temperatures lower than -20 °C in a dry and dark place.

TÜBİTAK UME cannot be held responsible for changes that might happen to the material at customer's premises due to noncompliance of the instructions for use, and the storage conditions given in the certificate.

Safety Information

Raw material: Donor horse serum (origin: Germany) was tested by the manufacturer (Biochrom) for mycoplasma and sterile filtered (0.1 µm). This substance is not classified as dangerous according to Directive 67/548/EEC. Complete toxicity testing was not performed. The usual laboratory safety measures apply. The material is suitable for *in-vitro* use only. Safety Data Sheet (SDS) should be read before use.

Participants

The laboratory that participated to the characterization study is given in the table below.

Laboratory	Address
TÜBİTAK UME	TÜBİTAK Gebze Yerleşkesi, Barış Mahallesi, Dr. Zeki Acar Caddesi No.1, 41470 Gebze - Kocaeli / Türkiye

Methods and/or Techniques Used for the Determination of the Certified Values

Methods and techniques used in characterization studies are given below.

Method/Technique	Parameter
Isotope Dilution Liquid Chromatography Mass Spectrometry (ID-LC-MS)	25-hydroxy-vitamin D_2 and 25-hydroxy vitamin D_3

Commutability

The intended use of this reference material is to check method performance and validation of 25-hydroxy vitamin D_2 and 25-hydroxy vitamin D_3 measurements in serum with LC-MS, LC-MS/MS and HPLC-UV methods.

Commutability of the material with routine *in vitro* diagnostic immunoassay methods has not been assessed. The user would need to assess the commutability, if UME CRM 1308 is going to be used as calibrant for routine *in vitro* diagnostic immunoassay methods.

Revision History

Date	Remarks
09.01.2015	First Issue
05.03.2015	Editorial changes
17.06.2015	Editorial changes
14.08.2015	Participants list is updated to include just the laboratory who participated in the characterisation study.
31.08.2016	Material is recertified with a new characterisation study. Density information is added, certified value and uncertainty calculated in ng/mL unit is presented. Commutability and Scope of Application remarks were added to the "Intended Use" part.
22.10.2018	Certificate is updated due to format change of the document.
18.09.2019	Information about shipping conditions is added. Certificate is updated due to changes in the format of certificate for reference materials.