National Institute of Standards and Technology
U.S. Department of Commerce

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Standard Reference Material® 2970
Vitamin D Metabolites in Frozen Human Serum
(25-Hydroxyvitamin D_2 High Level)
CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for validating methods for determining vitamin D metabolites in human serum and plasma and qualifying control materials produced in-house and analyzed using those methods.

Description: A unit of SRM 2970 consists of two vials of one concentration level of frozen human serum. Each vial contains approximately 1.0 mL serum.

Certified Values: Certified values are provided in Table 1 and Table 2. A NIST certified value is the present best estimate of the true value. Values in Table 1 are metrologically traceable to the International System of Units (SI) through determinations of purity of the primary calibration standards. The value in Table 2 is metrologically traceable to the SI units of mass and length.

Table 1. Certified Values for Vitamin D Metabolites in SRM 2970

Measurand	Mass Fraction[a] (ng/g)	Mass Concentration[a,b] (ng/mL)	Amount Concentration[a,c] (nmol/L)
25-Hydroxyvitamin D_2 [25(OH)D_2]	23.0 ± 0.3	23.5 ± 0.3	56.9 ± 0.8
25-Hydroxyvitamin D_3 [25(OH)D_3]	9.42 ± 0.24	9.63 ± 0.31	24.0 ± 0.8
Total 25-hydroxyvitamin D[d]	32.4 ± 0.4	33.1 ± 0.4	81.0 ± 1.1

[a] Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.

[b] Mass concentration levels were calculated from mass fractions using measured serum density listed in Table 2.

[c] Amount concentration values, nmol/L, are calculated from the mass concentration results, nanogram per milliliter, via multiplication by $1000/M$, where M is the molar mass, grams per mol, of the analyte. The molar masses used are: $M_{25\text{-hydroxyvitamin D2}} = 412.647$ g/mol and $M_{25\text{-hydroxyvitamin D3}} = 400.636$ g/mol. These molar masses have associated standard uncertainty $u(M) = 0.016$ g/mol.

[d] Total 25-hydroxyvitamin D is based on the combination of the certified values for 25(OH)D_2 and 25(OH)D_3.

Table 2. Certified Serum Density of SRM 2970 at 23 °C[a]

Measurand	Value (g/mL)
Serum Density at 23 °C	1.0223 ± 0.0015

[a] Value is expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$.

Period of Validity: The certified values delivered by **SRM 2970** are valid within the measurement uncertainty specified until **01 April 2029**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. The certifications are nullified if the SRM is damaged, contaminated, or otherwise modified.

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Maintenance of Certified Values: NIST will monitor this SRM to the end of the period of validity. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Safety: SRM 2970 IS INTENDED FOR RESEARCH USE. This is a human-source material. Handle product as a biohazardous material capable of transmitting infectious disease. The supplier has reported that each donor unit of serum used in the preparation of this product was tested by FDA-licensed tests and found to be non-reactive for human immunodeficiency virus (HIV), HIV-1 and HIV-2, hepatitis B surface antigen, and hepatitis C. However, no known test method can offer complete assurance that hepatitis B virus, hepatitis C virus, HIV, or other infectious agents are absent from this material. As such, this human blood-based product should be handled at Biosafety Level 2 as recommended by the Centers for Disease Control and Prevention's Biosafety in Microbiological and Biomedical Laboratories (6th edition) for human-derived blood products where the presence of an infectious agent may be unknown.

Source: This SRM was developed after an appropriate human subjects research determination.

Storage: Until required for use, original unopened vials of SRM 2970 should be stored in the dark at or below –70 °C.

Use: SRM 2970 is provided as a set of two vials of frozen serum. The vial (or vials) to be used should be allowed to thaw at room temperature for at least 30 min under subdued light. The contents of the vial should then be gently mixed prior to removal of a test portion for analysis. Precautions should be taken to avoid exposure to strong UV light and direct sunlight. The certification only applies to the initial use and the same results are not guaranteed if the remaining material is used at a later date.

Additional Information: Support for the development of SRM 2970 was provided in part by the National Institutes of Health Office of Dietary Supplements. Full details on the production, analysis, and statistical evaluation of SRM 2970 are provided in NIST Special Publication 260-210.

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sharpless, K.E.; Sieber, J.R.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf (accessed Aug 2021).

[2] Hahm, G.; Nelson, M.; Camara, J.; Toman, B.; *Certification of Standard Reference Material® s 2969 and 2970: Vitamin D Metabolites in Frozen Human Serum (Total 25-Hydroxyvitamin D Low Level) and (25-Hydroxyvitamin D_2 High Level)*; NIST Special Publication 260-210 (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-210.pdf (accessed Aug 2021).

If you use this SRM in published work, please reference:

Hahm G, Nelson M, Camara J, Toman B (2021) Certification of Standard Reference Material®s 2969 and 2970: Vitamin D Metabolites in Frozen Human Serum (Total 25-Hydroxyvitamin D Low Level) and (25-Hydroxyvitamin D_2 High Level). (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) 260-210. https://doi.org/10.6028/NIST.SP.260-210

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