NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

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Standard Reference Material® 1955a
Homocysteine in Frozen Human Serum
CERTIFICATE OF ANALYSIS

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

Description: A unit of SRM 1955a consists of one vial each of three materials: Level I, Level II, and Level III Each vial contains approximately 1.1 mL of frozen human serum.

Certified Values: Metrological traceability to the International System of Units (SI) of the assigned homocysteine values in the SRM 1955a materials is through calibration with a neat DL-homocysteine that was purity assessed by ^{1}H-qNMR through determinations traceable to the NIST PS1 Primary Standard for qNMR (Benzoic Acid). These certified values are traceable to the SI unit of mass. The values are reported on an as-received basis [1–3].

Table 1. Certified Values for Homocysteine in SRM 1955a

	Mass Fraction[a] (µg/g)	Mass Concentration[a,b] (µg/mL)	Amount Concentration[a,c] (µmol/L)
Level I	0.746 ± 0.018	0.759 ± 0.018	5.62 ± 0.14
Level II	1.383 ± 0.041	1.415 ± 0.042	10.47 ± 0.30
Level III	1.983 ± 0.028	2.028 ± 0.028	15.00 ± 0.21

[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 values were calculated from mass fractions using measured serum density listed in Table 2.

[c] Amount concentration values, micromole per liter, are calculated from the mass concentration results, microgram per milliliter, via multiplication by 1000/ $M_{\text{homocysteine}}$, where $M_{\text{homocysteine}}$ (molar mass expressed as grams per mol) is equal to 135.187 g/mol with an associated standard uncertainty of $u(M)$ = 0.006 g/mol.

Table 2. Certified Values of Serum Density for SRM 1955a at 23 °C

	Density[a] (g/mL)
Level I	1.01809 ± 0.00036
Level II	1.02307 ± 0.00038
Level III	1.02284 ± 0.00080

[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 $U_{95\%}(x)/2$.

Period of Validity: The certified values delivered by **SRM 1955a** are valid within the measurement uncertainty specified until **31 March 2029**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

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Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

Safety: SRM 1955a IS INTENDED FOR RESEARCH USE. This is a human-source material. SRM 1955a is a Biosafety Level 2 material and should be handled according to applicable federal, state, and/or local regulations and according to policies and procedures of recipient's organization. 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 negative for human immunodeficiency virus (HIV), HIV-1 antigen, hepatitis B, surface antigen, and hepatitis C.

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

Storage: The serum is shipped frozen (on dry ice) and, upon receipt, should be stored at –80 ˚C until ready for use. The SRM should not be exposed to sunlight or ultraviolet radiation. Storage of thawed material at room or refrigerator temperatures may result in changes in homocysteine concentrations.

Use: SRM 1955a is provided as a set of three vials of frozen human serum. The vial (or vials) to be analyzed should be removed from the freezer and allowed to stand at room temperature (20 °C to 25 °C) until thawed. After the material is thawed to room temperature, it should be used immediately. The material should be swirled gently to mix it before aliquots are withdrawn.

Additional Information: Full details on the production, analysis, and statistical evaluation of SRM 1955a are provided in NIST Special Publication 260-247 [4].

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.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; 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, 2021 edition; National Institute of Standards and Technology, Gaithersburg, MD (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Jun 2024).

[2] Nelson, B.C.; Pfeiffer, C.M.; Sniegoski, L.T.; Satterfield, M.B.; *Development and Evaluation of an Isotope Dilution LC/MS Method for the Determination of Total Homocysteine in Human Plasma*; Anal. Chem., Vol. 75, pp. 775–784 (2003).

[3] Satterfield, M,B.; Sniegoski, L.T.; Michael, J.; Welch, M.J.; Nelson, B.C.; *Comparison of Isotope Dilution Mass Spectrometry Methods for the Determination of Total Homocysteine in Plasma and Serum;* Anal. Chem., Vol. 75, pp. 4631–4638 (2003).

[4] Wood, E.S.C; Burdette, C.Q.; Mulloor, J.; Nelson, M.A.;, Urbas, A.A.;, Toman, B.; *Certification of Standard Reference Material® 1955a Homocysteine in Frozen Human Serum*; NIST Special Publication 260-247; National Institute of Standards and Technology, Gaithersburg, MD (2024); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-247.pdf (accessed Jun 2024).

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

Burdette CQ, Wood ESC, Mulloor J, Nelson MA, Urbas AA, Toman B.; (2024) Certification of Standard Reference Material® 1955a Homocysteine in Frozen Human Serum. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) 260-247. https://doi.org/10.6028/NIST.SP.260-247

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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