

Standard Reference Material® 956e

Electrolytes in Frozen Human Serum

CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for the calibration and validation of procedures and methods employed in clinical analysis for the determination of electrolytes in either diluted or undiluted human serum or plasma [1].

Description: A unit of SRM 956e consists of six sealed borosilicate glass ampoules of frozen human serum, two ampoules each of three different concentration levels: Level 1, Level 2, and Level 3. Each ampoule contains approximately 2 mL of frozen human serum.

Certified Values: NIST certified values are traceable to the International System of Units (SI) derived unit of amount concentration and mass concentration, expressed as millimoles per liter and milligrams per deciliter, respectively [2].

Electrolyte	Amount Concentration ^(a) (mmol/L)	Mass Concentration ^(a) (mg/dL)
Level 1		
Calcium (Ca)	3.030 ± 0.024	12.144 ± 0.098
Chloride (Cl ⁻)	98.07 ± 0.21	347.66 ± 0.74
Lithium (Li)	2.080 ± 0.017	1.444 ± 0.012
Magnesium (Mg)	1.463 ± 0.020	3.555 ± 0.049
Potassium (K)	5.978 ± 0.068	23.37 ± 0.26
Sodium (Na)	122.48 ± 0.53	281.6 ± 1.2
Level 2		
Calcium (Ca)	2.515 ± 0.031	10.08 ± 0.12
Chloride (Cl ⁻)	110.91 ± 0.22	393.19 ± 0.77
Lithium (Li)	1.303 ± 0.027	0.905 ± 0.019
Magnesium (Mg)	0.9905 ± 0.0097	2.407 ± 0.024
Potassium (K)	3.969 ± 0.046	15.52 ± 0.18
Sodium (Na)	141.98 ± 0.52	326.4 ± 1.2
Level 3		
Calcium (Ca)	2.026 ± 0.017	8.122 ± 0.070
Chloride (Cl ⁻)	125.26 ± 0.27	444.03 ± 0.94
Lithium (Li)	0.530 ± 0.013	0.3678 ± 0.0093
Magnesium (Mg)	0.5270 ± 0.0076	1.281 ± 0.018
Potassium (K)	1.998 ± 0.023	7.811 ± 0.089
Sodium (Na)	162.16 ± 0.56	372.8 ± 1.3

^(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. $U_{95\%}(x)$ is calculated such that the interval $x \pm U_{95\%}(x)$ is a 95 % confidence interval for the true value of the analyte. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [3–7].

Non-Certified Values: Non-certified values for ionized calcium, phosphorus and density are provided in Appendix A.

Period of Validity: The certified values delivered by **SRM 956e** are valid within the measurement uncertainty specified until **31 December 2034**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

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 956e IS INTENDED FOR RESEARCH USE. This is a human-source material. SRM 956e 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 of this serum has reported that each donor unit of serum or plasma used in the preparation of this product has been tested by FDA-approved methods and found to be non-reactive/negative for human immunodeficiency virus (HIV) 1 and 2 antibodies, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), and syphilis.

Storage: Original unopened ampoules of SRM 956e are shipped frozen (on dry-ice) and, upon receipt, shall be immediately stored between $-50\text{ }^{\circ}\text{C}$ and $-80\text{ }^{\circ}\text{C}$ until needed for use. A storage temperature of $-20\text{ }^{\circ}\text{C}$ is acceptable for storage up to one week before use. The SRM should not be exposed to sunlight or ultraviolet radiation.

Use: For analysis of all electrolytes except ionized calcium: Each ampoule should be inspected carefully for circular cracks at the base. If the ampoule is cracked or has visible deposits of serum material on the outside, it shall not be used. The serum in intact ampoules shall be thawed to room temperature and mixed by inverting gently at least five times before sampling. Check that all of the liquid has drained out of the neck of the ampoule. If needed, gently tap the neck to facilitate drainage. **NOTE THAT AMPOULES ARE NOT PRESCORED.** When opening ampoules, wear appropriate eye protection, protective gloves, and protective clothing. To open, score around the narrowest part of the neck with a file or other suitable device, and snap open. Once opened, the test portions shall be sampled as soon as possible. Any remaining materials should be discarded. A minimum sample size of 0.1 mL should be used.

For analysis of ionized calcium: Because pH influences ionized calcium, it is important that the samples be thawed and re-equilibrated with the gas in the ampoule headspace using the specified conditions given below [8].

1. Remove samples from freezer and thaw at ambient temperature for 1 hour and 40 minutes. NOTE: Ambient temperature must be between $20\text{ }^{\circ}\text{C}$ and $24\text{ }^{\circ}\text{C}$.
2. During the first few minutes of thawing, inspect ampoules carefully for cracks or breaks. Ampoules that are cracked or broken shall not be used and should be appropriately discarded.
3. After the 1 hour and 40 minutes thawing period, shake each ampoule vigorously with an up and down motion along the cylindrical axis for 10 seconds to create foam.
4. Wait an additional 30 minutes after shaking, then begin analyzing the samples.
5. Open the ampoule and aspirate the sample from as close as possible to the bottom of the ampoule. The sample must be introduced into the analyzer within one minute of opening the ampoule.
6. If it is not possible to aspirate sample directly from the ampoule into the analyzer for the particular system being used, the sample may be aspirated into a syringe while minimizing contact with air. NOTE: The sample shall be analyzed within one minute of opening the ampoule.

REFERENCES

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

Yu L, Barber C, Christopher S, Easley R, Johnson M, Murphy K, Vetter T, Waters J, Wood L (2025) Certification of Standard Reference Material® 956e Electrolytes in Frozen Human Serum. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) 260-254. <https://doi.org/10.6028/NIST.SP.260-254>

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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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APPENDIX A

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not meet the NIST criteria for certification [2] or provide metrological traceability to the International System of Units (SI) or other higher-order reference system. The values are provided with an uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods. Non-certified concentration values for phosphorus and ionized calcium are provided below.

Electrolyte	Molar Concentration ^(a) (mmol/L)	Mass Concentration ^(a) (mg/dL)
Level 1		
Ionized Calcium (Ca ²⁺)	1.743 ± 0.082	6.99 ± 0.33
Phosphorus (P)	4.254 ± 0.073	13.18 ± 0.23
Level 2		
Ionized Calcium (Ca ²⁺)	1.42 ± 0.11	5.67 ± 0.45
Phosphorus (P)	3.666 ± 0.073	11.36 ± 0.23
Level 3		
Ionized Calcium (Ca ²⁺)	1.116 ± 0.053	4.47 ± 0.21
Phosphorus (P)	3.08 ± 0.25	9.53 ± 0.77

^(a) Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. $U_{95\%}(x)$ is calculated such that the interval $x \pm U_{95\%}(x)$ is a 95 % confidence interval for the true value of the analyte [3–7].

Density: The mean non-certified densities and expanded uncertainties at 22 °C determined by the Lang-Levy Micropipette method are provided to allow conversions between results expressed in mass fraction and mass concentration [9]. The density values are 1.02215 g/mL ± 0.00023 g/mL for Level 1, 1.02242 g/mL ± 0.00018 g/mL for Level 2, and 1.02300 g/mL ± 0.00013 g/mL for Level 3. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. $U_{95\%}(x)$ is calculated such that the interval $x \pm U_{95\%}(x)$ is a 95 % confidence interval for the true density value [5].

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix 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>).

***** End of Appendix A *****