

Standard Reference Material[®] 2372a

Human DNA Quantitation Standard

CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended for use in the value assignment of human genomic deoxyribonucleic acid (DNA) quantitation materials, primarily those used for quantitative polymerase chain reaction (qPCR).

Description: A unit of SRM 2372a consists of three well-characterized human genomic DNA materials in pH 8.0 aqueous buffer. The components are derived from human buffy coat samples and labeled A, B, and C. Component A consists of genomic DNA from a single male donor. Component B consists of genomic DNA from a single female donor. Component C consists of a gravimetric mixture of genomic DNA (1 part male donor to 3 parts female donor). A unit of the SRM consists of one sterile 0.5 mL vial of each component, each vial containing approximately 55 μ L of DNA solution. Each of these vials is labeled and is sealed with a color-coded screw cap.

Certified Values: Certified values are provided in Table 1. These certified values were determined based on droplet digital polymerase chain reaction (ddPCR) assay counts of ten unique targets on eight chromosomes, dilution factors, and droplet volume measurements. A NIST certified value is a value for which NIST has the highest confidence in that all known or suspected sources of bias have been accounted for.

Table 1. Certified Values of Number and Mass Concentration for SRM 2372a^(a)

The copy number values are metrologically traceable to the natural units count 1 and ratio 1 and International System of Units (SI) derived units of volume. The DNA mass concentration values are metrologically traceable to the natural units count and ratio 1 and SI derived units of mass and volume.

Component	Copy Number ^(b) (per nL)	DNA ^(c) (ng/ μ L)
A (red cap)	15.1 \pm 1.5	49.8 \pm 5.0
B (white cap)	17.5 \pm 1.8	57.8 \pm 5.8
C (blue cap)	14.5 \pm 1.5	47.9 \pm 4.8

^(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) Copy number concentration values, human haploid genome equivalents (HHGE) per nanoliter, are based on droplet digital (ddPCR) assay counts of ten unique targets on eight chromosomes, dilution factors, and droplet volume measurements.

^(c) Mass concentration values, nanograms of human genomic DNA per microliter, are calculated from the number concentration results, the number of nucleotide base pairs (bp) in the reference HHGE, and the mean molecular weight of the sodium salts of the nucleotide monomers that comprise the DNA polymer.

Expiration of Certification: The certification of **SRM 2372a** is valid, within the stated measurement uncertainty, until **13 February 2023**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Storage and Handling”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Non-Certified Values: Table 2 lists values for the number of mitochondrial genomes (mtDNA) relative to the number of nuclear haploid genomes (nDNA) present in the SRM 2372a components. Because they are based on a limited number of independent ddPCR assays, these values do not meet NIST's criteria for certification but are the best currently available estimates.

Table 2. Assay-Specific mtDNA/nDNA Ratios^(a)

These values are metrologically traceable to the ddPCR measurement method and assays used.

Component	mtDNA/nDNA
A (red cap)	174 ± 4
B (white cap)	206 ± 5
C (blue cap)	279 ± 7

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

Storage and Handling: Until required for use, SRM 2372a should be stored in the dark between 2 °C and 8 °C. Component vials should be mixed briefly and centrifuged (without opening the vial cap) prior to removing sample aliquots for analysis. For the certified values to be applicable, materials should be withdrawn immediately after opening the vials and processed without delay. Certified values do not apply to any material remaining in recapped vials. 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.

Use: For qPCR assays, value assign secondary standard DNA solutions relative to the certified mass concentrations. Calibrate the assays to SRM 2372a using one or more dilution series prepared from a SRM 2372a component. For non-gender-specific qPCR assays, all three components should be used as calibrants to elucidate potential material-assay specific interactions. Users should keep in mind that a potential for pipetting error exists; therefore, suitable care should be exercised in preparing calibration solutions.

Safety: Each donor unit used in the preparation of this product was tested by FDA-licensed tests and found to be negative for human immunodeficiency virus (HIV-1 RNA and Anti-HIV 1/2), hepatitis B (HBV DNA and HBsAg), hepatitis C (HCV RNA and Anti-HCV), West Nile virus (WNV RNA) and syphilis. Additionally, each donor was tested according to FDA guidelines for *Trypanosoma cruzi* (Chagas) and found to be negative/non-reactive. However, no known test method can offer complete assurance that infectious agents are absent from this material. Accordingly, this human blood-based product should be handled at the Biosafety Level 1. SRM 2372a components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

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

Additional Information: Full details on the production, analysis, and statistical evaluation of SRM 2372a are provided in: NIST Special Publication 260-189, *Certification of Standard Reference Material® 2372a Human DNA Quantitation Standard*. This publication is available free of charge at <https://doi.org/10.6028/NIST.SP.260-189>.

NIST will monitor this SRM until its certification expires. If substantive technical changes occur that affect the certified values before this certificate expires, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. Contact the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, Maryland 20899-2300; telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or the internet at <https://www.nist.gov/srm>.