National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2372

Human DNA Quantitation Standard

Standard Reference Material (SRM) 2372 is intended primarily for use in the value assignment of human genomic deoxyribonucleic acid (DNA) forensic quantitation materials. It is not intended for any human or animal clinical diagnostic use. SRM 2372 consists of three well-characterized human genomic DNA materials solubilized in 10 mmol/L 2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride (Tris HCl) and 0.1 mmol/L ethylenediaminetetraacetic acid disodium salt (disodium EDTA) using deionized water adjusted to pH 8.0 buffer (TE^{-4}). The three component genomic DNA materials, labeled A, B, and C, are derived from a single male donor, multiple female donors, and multiple male and female donors, respectively. A unit of the SRM consists of one sterile 2 mL vial of each component, each vial containing approximately 110 µL of DNA solution. Each of these vials is labeled and is sealed with a color–coded screw cap.

Certified Values: Table 1 lists the "apparent absorbance," $A_{260–320}$, for component materials diluted with an equal volume of 0.4 mol/L aqueous sodium hydroxide (NaOH) and measured against TE^{-4} mixed with an equal volume of 0.4 mol/L NaOH solution. The $A_{260–320}$ values are calculated as the decadic attenuance, D_{10}, at 260 nm minus the D_{10} at 320 nm with an optical pathlength of 1.0 cm, spectral bandwidth of 1 nm, and a temperature of 22 ºC ± 1 ºC. The D_{10} values are indicated by the absorbance scale of the spectrophotometer. The D_{10} scale is unitless. Each apparent absorbance is expressed as a certified value ± expanded uncertainty, where the expanded uncertainty is approximately 2.03 times the standard uncertainty and is consistent with the ISO and NIST guides [1]. The certified value ± expanded uncertainty specifies an interval that is expected to include the true $A_{260–320}$ value of an accurately prepared equal volume DNA/NaOH mixture with approximately a 95 % level of confidence for any given SRM 2372 vial [2]. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [2]. The uncertainty intervals are estimated from the evaluation of two independent sets of 18 vials of each component mixed with an equal volume of 0.4 mol/L NaOH. Treatment with NaOH forces the DNA solutions to have a single-stranded conformation and enables conversion of the $A_{260–320}$ values to a conventional estimate of DNA concentration [3].

Table 1. Certified Apparent Absorbance ($A_{260} – A_{320} = D_{10}$ at 260 nm – D_{10} at 320 nm) Values for Components Treated with NaOH

Component A	Component B	Component C
0.777 ± 0.060	0.821 ± 0.095	0.804 ± 0.068

Expiration of Certification: The certification of **SRM 2372** is valid**,** within the measurement uncertainty specified, until **31 December 2017,** provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

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Certificate Issue Date: 08 January 2013
Certificate Revision History on Last Page

Robert L. Watters, Jr., Director
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Information Values: Table 2 lists the $A_{260–320}$ values for the untreated components and an estimate of the DNA mass concentration of the SRM 2372 components based on the single–stranded conformation. These conventional concentration values are derived from the assertion that a solution of single–stranded DNA (ssDNA) with an absorbance of 1.0 at 260 nm and a pathlength of 1.0 cm has a DNA mass concentration of 37 µg/mL (37 ng/µL) [4]. An information value is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess the uncertainty associated with the value or only a limited number of analyses were performed [2].

Table 2. Information Values for Untreated Components

Component	Conventional ssDNA Concentration (ng/µL)	$A_{260–320}$
A	57	1.2
B	61	1.3
C	59	1.3

Coordination of the technical measurements leading to this certification was under the direction of M.C. Kline and J.M. Butler of the NIST Biomolecular Measurement Division. Evaluation of the data was performed by D.L. Duewer of the NIST Chemical Sciences Division. Evaluation of the measurement uncertainty was performed by H-k. Liu of the NIST Statistical Engineering Division.

Sample preparation and analytical measurements were by NIST Biomolecular Measurement Division staff: M.C. Kline and J.W. Redman prepared the samples; M.C. Kline made the D_{10} measurements and assessed the NaOH concentration and temperature dependence of the DNA/NaOH mixtures; and E.L.R. Butts evaluated the performance of the components in selected quantitative polymerase chain reaction (qPCR) assays. An interlaboratory study that assessed the commutability of the SRM among various qPCR assays was coordinated by M.C. Kline.

Support aspects involved in the preparation and issuance of this SRM were coordinated through the NIST Office of Reference Materials.

The preparation of this SRM was supported in part by the National Institute of Justice, U.S. Department of Justice.

NOTICE AND WARNING TO USERS

SRM 2372 IS A HUMAN-SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE THE SRM 2372 COMPONENTS AS BIOSAFETY LEVEL 1 MATERIALS CAPABLE OF TRANSMITTING INFECTIOUS DISEASE [5]. SRM 2372 components and derived solutions should be disposed of in accordance with local, state, and federal regulations.

INSTRUCTIONS FOR STORAGE AND USE

Storage: All vials of SRM 2372 should be stored in the dark between 2 °C to 8 °C. **DO NOT FREEZE.**

DO NOT EXPOSE ANY DNA SOLUTION TO DIRECT SUNLIGHT. Component vials should be mixed briefly and centrifuged (without opening the vial cap) prior to removing sample aliquots for analysis. For the certified and informational values to be applicable, materials should be withdrawn immediately after opening the vials and processed without delay. Certified and information values do not apply to any material remaining in recapped vials. There is no minimum sample size requirement, the amount required by any given assay will be dictated by the nature of the assay.

For use with qPCR assays: When used with qPCR assays, do not dilute the DNA materials with NaOH solution. Use the provided information-value concentrations to value assign secondary standard DNA solutions for routine use. Users may calibrate their assays to SRM 2372 using one or more dilution series, with each series prepared from one SRM 2372 component. For non-gender-specific qPCR assays, all three components should be used as calibrants to elucidate potential material-assay specific interactions. Component C should NOT be used to calibrate gender-specific assays, since it is derived from a mixture of unknown proportions of male and female donors. Users should keep in mind that a potential for pipetting error exists; therefore, suitable care should be exercised in preparing calibration solutions. See reference 6 for further information.

For use with spectrophotometric assays: Equal volumes of a DNA material and a freshly prepared 0.4 mol/L NaOH solution should be combined, mixed briefly, and centrifuged prior to measurement. An appropriate

spectroscopic blank is prepared by mixing equal volumes of TE^{-4} and the 0.4 mol/L freshly prepared NaOH solution. The DNA/NaOH solutions are stable for at least 1 h. Other dilution protocols may be used as required but they must yield a final NaOH concentration of approximately 0.2 mol/L. Based on Annex B of ISO 21571 [1] the conventional DNA concentration ([DNA]) of the samples is:

$$[\text{DNA}] = F \times (D_{10} \text{ at } 260 \text{ nm} - D_{10} \text{ at } 320 \text{ nm}) \times 37$$

where

F is the dilution factor and for equal-volume dilutions is equal to 2,
D_{10} is the spectrophotometric absorbance indication at the specified wavelength,
37 is the conventional conversion factor for ssDNA as ng/µL or µg/mL.

Figure 1 displays the average apparent absorbance spectra, D_{10} at 220 nm to 320 nm minus D_{10} at 320 nm, for the three SRM 2372 components. The dashed lines are the spectra for the materials as delivered in the vials, referenced to TE^{-4}, pH 8.0 buffer. The solid lines are the spectra for the materials diluted with an equal volume of 0.4 mol/L NaOH, referenced to TE^{-4} diluted with an equal volume of 0.4 mol/L NaOH.

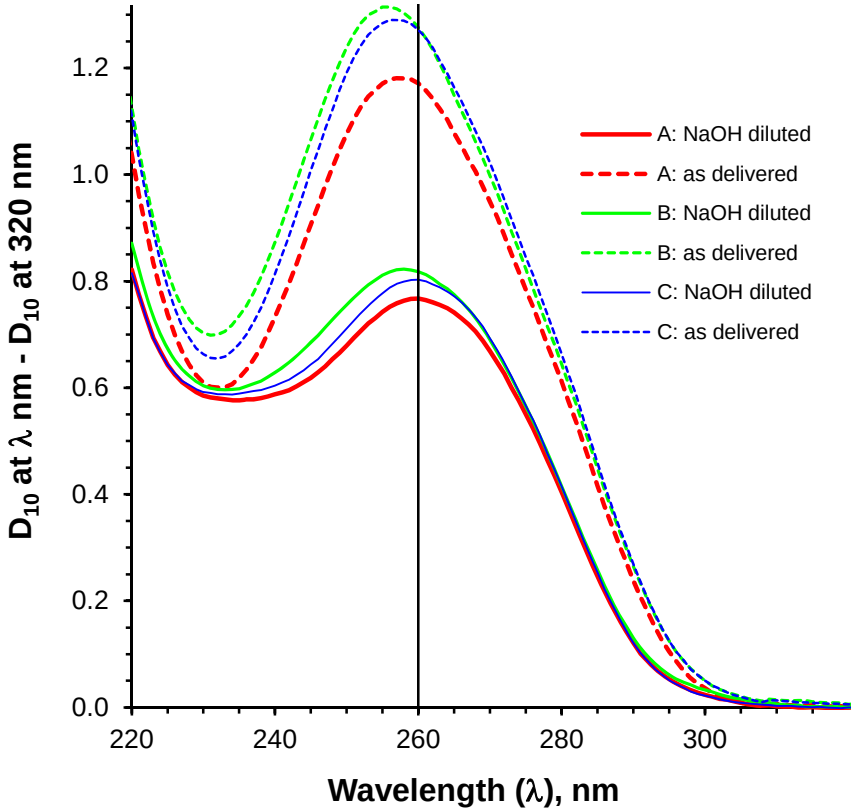

Figure 1. Apparent Absorbance

Note: 0.2 mol/L NaOH solutions are caustic. It may be more appropriate for users of micro–volume spectrophotometers that do not isolate sample solutions in non–reactive optical chambers to evaluate the untreated materials and compare the results to the information $A_{260–320}$ values.

Spectroscopic Traceability: When used for spectroscopic value assignment of secondary DNA quantitation standards, a research-quality dual-beam spectrophotometer must be used that can achieve a spectral bandwidth of 1.0 nm and can control the sample temperature at 22 ºC ± 1 ºC. The wavelength calibration of the instrument should be verified using a suitable holmium oxide solution standard [7]. The instrument must reliably measure $A_{260–320}$ in solutions of volume 0.1 mL or less. This capability can be verified using a suitable absorption standard [8]. The candidate secondary DNA quantitation standard(s) must be prepared in TE^{-4}. In dual–beam mode, optically matched cuvettes can be used to directly compare an unknown solution with a given SRM 2372 component. Indirect comparisons can be accomplished with the buffer that was used to prepare the candidate secondary standard(s) as the reference blank for both the SRM and candidate materials.

PREPARATION AND ANALYSIS[1]

Components A and B: Human buffy coats from anonymous donors were obtained from a commercial source. The method used to isolate genomic DNA from the white blood cells found in the human buffy coats is based on a modified "salting out" procedure [9]. After the initial extraction, the material was re-extracted to assure purity. Component A was treated with bovine pancreatic ribonuclease A before re-extraction [4]. Extracted DNA for components A and B was air–dried in a laminar flow hood and stored in perfluoroalkoxy fluoropolymer (PFA) containers at 4 °C prior to solubilization in TE^{-4}. After addition of buffer to the air–dried DNAs in their PFA containers, the materials were allowed to equilibrate at 4 °C until there was complete visual disappearance of solids (several days to weeks, depending on the material). After concentration adjustment, the solubilized materials were stored in their PFA containers at 4 °C until vialing. Just prior to vialing, each material was brought to room temperature in its PFA container inside a laminar flow hood. The solution was gently mixed. Each solution was transferred in turn to pre–labeled sterile vials with an automatic pipette, and the vials were capped. After the production run for that component was complete, all vials were placed into storage at 4 °C.

Component C: Purified unsheared genomic human DNA was purchased from Sigma–Aldrich Co., St. Louis, MO. Literature accompanying the material indicated that the material was purified by equilibrium buoyant density ultracentrifugation in cesium chloride, dialyzed against a solution of 1 mmol/L sodium chloride, 1 mmol/L disodium EDTA, and 1 mmol/L Tris HCl, pH 7.5, and then lyophilized. Solubilization, storage, and vialing of this material was the same as described above for components A and B.

Temperature and NaOH Concentration Dependence: For all three components, the $A_{260–320}$ values change less than 0.001 per degree Celsius change in spectral acquisition temperature from 20 °C to 24 °C. The $A_{260–320}$ values change by approximately 0.01 per 0.1 mol/L NaOH from final NaOH concentrations of 0.1 mol/L to 0.3 mol/L.

Commutability of the DNA Mass Concentration Information Values: The commutability of the three SRM 2372 components in commonly used DNA quantitation assays was assessed by an interlaboratory study involving 32 participants and 11 non–gender–specific methods. While the relative results for the three components for most methods agreed within the method reproducibilities, systematic differences in response were observed with specific qPCR methods. These biases were confirmed by studies at NIST. The observed among-method variability is not unexpected since different qPCR methods exploit qualitatively different molecular targets. For information on the performance of the SRM 2372 components with selected qPCR methods, see reference 6.

[1] Certain commercial equipment, instruments, or materials are identified in this certificate 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.

REFERENCES

[1] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (ISO GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Jan 2013); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/ (accessed Jan 2013).

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Certificate Revision History: 08 Jan 2013 (Re-certification using an apparent absorbance method; extension of the certification period; editorial changes); **18 September 2007** (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.