



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2366a

Cytomegalovirus DNA (Towne_{Δ147} BAC) for DNA Measurements

This Standard Reference Material (SRM) is intended for use in the value assignment of the number of amplifiable genome copies of cytomegalovirus (CMV) per volume sample. When used to value assign practical CMV calibration materials, SRM 2366a can provide traceability to the International System of Units (SI) for measurement results of CMV viral load in tissues or fluids such as plasma. A unit of SRM 2366a consists of one sterile, 0.5 mL perfluoroalkoxy (PFA) fluoropolymer vial containing approximately 150 μ L of extracted DNA solubilized in TE buffer, pH 8.0. TE buffer consists of 10 mmol/L 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) and 1 mmol/L ethylenediaminetetraacetic acid (EDTA) tetrasodium salt in deionized water. The CMV DNA is in the form of a bacterial artificial chromosome – known as CMV Towne_{Δ147} BAC – that contains the genome of the Towne strain of CMV [1,2].

Table 1. Certified CMV Copy Number Values for SRM 2366a

Analyte	Certified Value, X	95 % Probability Uncertainty Interval	Effective Standard Uncertainty, $u(X)$	Effective Coefficient of Variation, $CV=100 \times u(X)/X$	Units
CMV copy number	1,796,000	1,746,000 to 1,849,000	26,000	1.5 %	Copies/ μ L

These certified values were determined as the consensus result from five polymerase chain reaction (PCR) assays using a droplet digital (ddPCR) system [3] and a direct measurement of the mean droplet volume. The certified value and its uncertainties were computed using a Gaussian random effect model [4]. 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 [5].

Uncertainty: The true value of the analyte in this material is expected to be within the given probability interval with about a 95 % level of confidence. For purposes of uncertainty propagation, use the stated $u(X)$ or CV, whichever is most convenient for your purpose; these values are associated with 60 degrees of freedom.

Traceability: The certified value is traceable to copies per volume, expressed as number of amplifiable CMV entities per microliters of solution, through the confirmation of identity, the ddPCR count measurements, the validity of the Poisson endpoint transformation of counts to entities for digital PCR endpoint assays [6], and calibrated volume measurements made at NIST. Metrological traceability is to the SI unit for volume.

Expiration of Certification: The certification of **SRM 2366a** is valid, within the measurement uncertainty provided, until **01 August 2020**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Handling, 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 or register online) will facilitate notification.

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Certificate Issue Date: 01 September 2015

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Coordination of the technical measurements leading to the certification was under the direction of M.C. Kline and P.M. Vallone of the NIST Biomolecular Measurement Division. R.J. Haynes prepared the materials. R.J. Haynes and E.L. Romsos performed measurements. Droplet volume measurements were provided by J.A. Dagata of NIST Semiconductor and Dimensional Metrology Division.

Evaluation of the data was performed by M.C. Kline, D.L. Duewer of NIST Chemical Science Division, and B. Toman of the NIST Statistical Engineering Division.

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

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling: SRM 2366a IS A VIRAL AND BACTERIAL DNA SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE SRM 2366a AS BIOSAFETY LEVEL 1 MATERIALS CAPABLE OF TRANSMITTING INFECTIOUS DISEASE [7]. SRM 2366a and derived solutions should be disposed of in accordance with local, state, and federal regulations.

Storage: Vials of SRM 2366a should be stored in the dark between 2 °C to 8 °C. **DO NOT FREEZE.**

Use: The vial should be mixed by inversion and centrifuged without opening the vial cap prior to removing sample aliquots for analysis. For the certified values to be valid, materials should be withdrawn immediately after opening the vials and processed without delay. Dilutions of these materials may be made as appropriate, but they must be used immediately. Certified values do not apply to any material remaining in recapped vials. **DO NOT EXPOSE ANY DNA SOLUTION TO DIRECT SUNLIGHT.**

PREPARATION AND ANALYSIS⁽¹⁾

Sample Preparation: The CMV Towne_{Δ147} BAC was obtained from the New Jersey Medical School (H. Zhu, Newark, NJ) and was propagated at NIST in *Escherichia coli* cells. Total DNA (CMV and *E. coli*) was isolated, purified, and re-suspended in TE buffer. After concentration adjustment, the material was allowed to equilibrate at 4 °C in PFA container until vialing. Just prior to vialing, the material was brought to room temperature inside a laminar flow hood and gently mixed. The solution was transferred to sterile PFA vials with an automatic pipette, which were then capped, labeled, and placed into storage at 4 °C.

Confirmation of CMV Identity: Sanger and Next Generation Sequencing (NGS) confirm that the CMV DNA in SRM 2366a is completely concordant with reference sequence AY315197.2.

PCR Assays: The five quantitative polymerase chain reaction assays used to characterize CMV copy number are listed in Table 2. The primer and probe sequences for these assays are listed in Table 3. Measurements were made using both ddPCR and chamber digital (cdPCR) systems.

Table 2. PCR Assays Used

Name	Locus	Nucleotide position ^(a) (bp)	Amplicon, (bp)	Assay source
CP1	UL54	78,453 to 78,524	72	[8]
gBA	UL56	82,475 to 82,728	254	[9,10]
IE	MIE	170,832 to 170,958	127	[11]
mie11	MIE	172,527 to 172,604	78	Modified from [12]
UL132	UL132	178,461 to 179,522 ^(b)	62	[13]

^(a) Relative to the CMV reference genome AY315197.2.

^(b) Commercial assay, position specified relative to the Merlin strain, CMV reference genome AY446894.2

⁽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.

Table 3. Primer and probe sequences.

Name	Type	Sequence
CP1	Forward primer ^(a)	GGCCGTTACTGTCTGCAGGA
	Reverse Primer ^(a)	GGCCTCGTAGTGAAAATTAATGGT
	Probe – BHQ	CCGTATTGGTGCGCGATCTGTTCAA
gBA	Forward primer ^(a)	TACCCCTATCGCGTGTGTTC
	Reverse Primer ^(a)	ATAGGAGGCGCCACGTATTC
	Probe – BHQ	TTGCTGCCCAGCAGATAAGTGGTG
IE	Forward primer ^(a)	CAAGCGGCCTCTGATAACCA
	Reverse Primer ^(a)	ACTAGGAGAGCAGACTCTCAGAGGAT
	Probe – BHQ ^(a)	TGCATGAAGGTCTTTGCCAGTACATTCT
mie11	Forward primer	GCATTGTGCTGTGCCTAAGTC
	Reverse Primer	ATGCCTCCAGCGGCTCATG
	Probe – BHQplus	TGGCCTCCACTGTTAGGA
UL132	20X assay – TaqMan MGB	Proprietary sequences

^(a) Per literature notation, but direction reversed relative to reference sequence AY315197.2

Homogeneity Assessment: Twenty-six units of SRM 2366a were selected randomly using stratified random sampling and the CMV copy number of their DNA solutions assessed with the CP1 assay using the ddPCR measurement. The dominant component of observed variance was within-unit repeatability. No systematic trend with production sequence was observed.

Short-Term Stability Assessment: Sixteen units of SRM 2366a were selected randomly using stratified random sampling and stored at –20 °C, 4 °C, 21 °C, and 37 °C (four vials at each temperature) for a period of 30 weeks. CMV copy numbers were assessed ten times during this period with the CP1 assay using both ddPCR and cdPCR measurement systems. The SRM 2366a CMV copy number is thermally stable at 4 °C, 22 °C, and 37 °C but is unstable to freeze-thaw cycling.

Certification Measurements: Seven units of SRM 2366a were selected randomly using stratified random sampling and the CMV copy number of their DNA solutions assessed with the five PCR assays listed in Tables 2 and 3 using both ddPCR and cdPCR measurement systems. Results were obtained from seven measurement campaigns over a period of eight months. Traceable results are currently available only for the ddPCR measurements. However, applying the manufacturer’s nominal chamber volume to the cdPCR measurements yields results in excellent agreement with the traceable ddPCR results. Thus the cdPCR measurements serve to validate the ddPCR results.

REFERENCES

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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>.