National Institute of Standards and Technology
U.S. Department of Commerce

Date of Issue:
24 March 2023

Standard Reference Material® 2365
BK Virus DNA Quantitative Standard
CERTIFICATE OF ANALYSIS

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

Description: SRM 2365 consists of a well-characterized, linearized plasmid, containing BK virus DNA solubilized in 10 mmol/L 2-amino-2-(hydroxymethyl)-1,3 propanediol hydrochloride (Tris HCl) and 1 mmol/L ethylenediaminetetraacetic acid disodium salt (disodium EDTA) pH 8.0 buffer (TE), with 50 ng/µL yeast tRNA added to ensure stability. A unit of the SRM consists of one 0.5 mL tube containing approximately 110 µL of DNA solution. The tube is labeled and is sealed with a screw cap.

Certified Values: Certified values are provided in Table 1. 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. 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 [1].

Table 1. Certified Values for SRM 2365

Analyte	Certified Value (copies/µL)	95% Probability Uncertainty Interval (copies/µL)	Standard Uncertainty, $u(X)$ (copies/µL)	Effective Coefficient of Variation, $CV=100\times u(X)/X$ (%)
BK Virus DNA copy number	558,000	534,000 to 582,000	12,000	2.2

Period of Validity: The certified values delivered by **SRM 2365** are valid within the measurement uncertainty specified until **15 July 2028**. 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).

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Safety: SRM 2365 is a bacterial DNA source material. Handle SRM 2365 as a Biosafety Level 1 material capable of transmitting infectious disease. SRM 2365 should be disposed to in accordance with local, state, and federal regulations.

Storage and Handling: Until required for use, SRM 2365 should be stored in the dark between 2 °C and 8 °C.

The screw-cap vial 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 copy number concentrations. Calibrate the assays to SRM 2365 using one or more dilution series prepared from SRM 2365. Users should keep in mind that a potential for pipetting error exists; therefore, suitable care should be exercised in preparing calibration solutions.

Source: The BK Virus genome construct (NCBI accession # JQ713822.1) was synthesized and cloned into a pUC57 plasmid. Restriction sites (AhdI and BssHII) were added on either side of the BK Virus DNA, to allow the BK genome region to be isolated from the rest of the plasmid. The plasmid was transformed into *E. coli* Sure2 cells. The plasmid was grown in a 2.5 L culture, purified, and linearized with restriction enzymes (AhdI and BssHII). The linearized material was then filtered to remove precipitate from bovine serum antigen (a component in the restriction digests). BK Virus DNA solution was combined with TE buffer, and 50 ng/µL yeast tRNA was added to ensure stability.

Additional Information: Full details on the production, analysis, and statistical evaluation of SRM 2365 are provided in NIST Special Publication 260-191, *Certification of Standard Reference Material® 2365 BK Virus DNA Quantitative Standard* [2].

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 Mar 2023).

[2] Cleveland, M.H.; Farkas, N.; Kiesler, K.M.; Toman, B.; Vallone, P.M.; *Certification of Standard Reference Material® 2365 BK Virus DNA Quantitative Standard.* NIST Special Publication 260-191, available at: https://doi.org/10.6028/NIST.SP.260-191 (accessed Mar 2023).

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

Cleveland, M.H.; Farkas, N.; Kiesler, K.M.; Toman, B.; Vallone, P.M (2018) Certification of Standard Reference Material® 2365 BK Virus DNA Quantitative Standard. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) 260-191. https://doi.org/10.6028/NIST.SP.260-191

Certificate Revision History: 24 March 2023 (Change of period of validity; updated format; editorial changes); **11 September 2018** (Original certificate date).

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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