ATCC® Catalog No. VR-3382™

Lentiviral Vector Reference Material (LVVRM) Produced by LVVRMWG

Description and background

The Lentiviral Vector Reference Material (LVVRM) is an HIV-1-based VSV-G pseudotyped Lentiviral vector encoding a green fluorescent protein (GFP) transgene. It is provided as a purified preparation formulated in sterile liquid 20mM HEPES, 75mM NaCl, and 2.5% sucrose (pH 7.0) and is stored frozen at -70°C. The configuration is 0.5 mL per cryovial.

The LVVRM was developed under the guidance of the Lentiviral Vector Reference Material Working Group (LVVRMWG), National Institute of Standards and Technology (NIST), Food and Drug Administration Center for Biologics Evaluation and Research (FDA/CBER), National Research Council Canada (NRC-Canada), American Type Culture Collection (ATCC), and BioProcessing Journal for use as a viral reference material when characterizing lentiviral gene therapy products. Information regarding the development and characterization of the LVVRM can be found on the *BioProcessing Journal*'s website at https://bioprocessingjournal.com/reference-materials/lentiviral-vector-reference-material-project/.

Characterization

Each lot of the LVVRM is thoroughly characterized to ensure quality, consistency, and safety for research and manufacturing applications. LVVRM characterization involves a diverse set of analytical assays designed to evaluate its structural integrity, biological activity, and safety profile.

The functional titer was measured by transducing HEK293 cells with the LVVRM and quantifying GFP expression, yielding a result of 2.3×10^7 transducing units per mL (TU/mL), which reflects the number of infectious particles capable of gene delivery. The physical titer, expressed both as lentiviral particles per mL (LP/mL) and transducing units per mL (TU/mL), was determined using a p24 ELISA assay, which quantifies the p24 capsid protein, a structural component of the lentiviral material. This assay reported 6.55×10^9 LP/mL. The genome copy number was established using droplet digital PCR (ddPCR), which provides absolute quantification of viral RNA genomes, resulting in 1.65×10^8 genome copies per mL.

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Table 1: Testing results for LVVRM lot 70075656

Test / Method	Result
Functional titer (Transduction of HEK293 cells with GFP readout)[1]	2.3 x 10^7 TU/mL
Physical Titer (p24 ELISA) Lentiviral Particles/mL (LP/mL)[2,3]	6.55 x 10^9 LP/mL
Residuals RNaseP (ddPCR)[4] HEK293 host cell protein (ELISA) Benzonase (EndonuclaseGTP ELISA)	Below limit of detection 196.012 ng/mL Below limit of detection
Genome Titer (ddPCR) Copies/mL	1.65 x 10^8 copies/mL
Replication-competent lentivirus (RCL) assay Amplification in C8166 cells; detection by p24 ELISA and lentiviral genome quantification (qPCR)	Negative

[1]Protocol adapted from addgene.org "Fluorescence Titering Assay for Lentivirus" Addgene: Fluorescence Titering Assay
[2]Each lentiviral particle is assumed to contain ~2,000 molecules of p24, corresponding to approximately 8 × 10^{-5} pg of p24 per particle, which allows conversion of p24 concentration to particle count using the factor 1 ng p24 ≈ 1.25 × 10^7 LPs
[3]It is assumed that 1 transducing unit (TU) corresponds to approximately 100–1000 LPs, allowing lentiviral titer (TU/mL) to be estimated
[4] ddPCR primers and probe targeted the Psi packaging signal region of the lentiviral vector genome

The LVVRM underwent comprehensive authentication and safety testing to ensure its identity and biosafety profile. Identity was confirmed by sequencing and western blot analysis targeting the p24 capsid. Mycoplasma contamination was ruled out using both broth and agar culture methods and PCR-based DNA detection, with no growth or DNA detected. Sterility was validated through 14-day incubations in BacT/ALERT 3D iAST (aerobic) and iNST (anaerobic) bottles at 32.5°C, both of which showed no microbial growth. Adventitious agent testing, including panels for human, mouse, and rat pathogens as well as next-generation sequencing (NGS), confirmed the absence of detectable contaminants. Additionally, endotoxin levels were measured using the chromogenic LAL assay and found to be low, measuring 0.186 EU/mL, well below the commonly accepted 0.5 EU/mL threshold for safety.

Recommended storage

Vials are shipped from ATCC on sufficient dry ice to maintain the product in frozen condition until received by the end user. Immediately upon receipt, store vials under frozen temperatures at -70°C to -90°C.

Stability data

LVVRM lots will undergo stability studies at 6 months, 1 year, and every 2 years thereafter.

Recommended use of the Lentiviral Vector Reference Material

The LVVRM is used to standardize lentiviral vector quantification and characterization in gene therapy development. It was developed to qualify and validate internal reference material that can subsequently be used in quantification assays such as particle concentration and infectious titer as well as to validate the infectious titer of the positive control virus used in replication-competent lentiviral assays. By calibrating potency assays and supporting assay qualification, the LVVRM ensures consistent vector dosing across laboratories. It serves as a common benchmark to improve study comparability, streamline regulatory submissions, and reduce variability in manufacturing and quality control, ultimately

accelerating the development of safe and effective lentiviral-based therapies.

Recommended host cells

The LVVRM was produced by chemical induction of a stable HEK293-based cell line (HEK293SF-LVP-GFP) grown in serum-free suspension culture. The producer cell line contained all genes necessary to produce lentiviral vectors: TR5-CuO-VSV-G (envelope protein), CMV-Gag/Pol, TR5-CuO-Rev, and Lentiviral vector (pCSII-CMV5-GFP). Transcription regulation was controlled by using cumate and doxycycline inducers.

Manufacture of the Lentiviral Vector Reference Material

The LVVRM is an HIV-1-based, VSV-G pseudotyped lentiviral vector that encodes a GFP transgene. This vector has been engineered to be replication-incompetent, ensuring that it does not replicate within the host cell. It retains the ability to deliver genetic material but lacks the machinery to produce new virus particles. This minimizes the chance of insertional mutagenesis, where multiple integrations could disrupt host genes. The inclusion of GFP enables easy tracking of transduction efficiency in vitro and in vivo. GFP fluoresces green under UV or blue light, allowing researchers to easily identify which cells have been successfully transduced by the vector. This enables real-time tracking of cellular processes like migration, division, or differentiation, without harming the cells or requiring additional staining.

The LVVRM was produced using a fed-batch bioreactor process optimized for high-titer output. A suspension-adapted HEK293 cell line (HEK293SF-LVP-GFP) that was engineered with an inducible expression system served as the production platform under serum-free conditions. This stable cell line was designed to express all necessary packaging components and a GFP-encoding transfer vector. Cultures were grown in bioreactors of varying volumes (4 L, 10 L, and 50 L) to assess scalability and consistency before proceeding with the production batch in a 100 L bioreactor. Sensors were used to measure and control temperature (37°C), pH (7.10 ± 0.05), dissolved oxygen (40%), and agitation. Induction of vector production was tightly regulated to minimize cytotoxicity and ensure process stability. Cultures were seeded at 45% (4-50 L bioreactors) or 57% (100 L bioreactor) of the final volume and expanded through dilution upon reaching cell densities of $\geq 1.5\times10^{6}$ cells/mL. This was followed by two feed additions, with the second feed introduced at $\geq 3.5\times10^{6}$ cells/mL after which induction was initiated. Final titers ranged from 1×10^{7} to 1×10^{8} transduction units per milliliter, with consistent performance across different reactor configurations.

Following production, the LVVRM was purified using a standardized downstream process. The Benzonase®-treated harvest underwent clarification by filtration to remove cellular debris and potential contaminants. The vector was then purified by Anion-Exchange chromatography, followed by concentration and formulation for stability, ensuring consistent quality across batches. Final material was shipped under controlled conditions to ATCC for aliquoting and distribution. This purification strategy was designed to support reproducibility and regulatory alignment, enabling the reference material to serve as a benchmark for assay validation and product comparability in gene and cell therapy development.

References

1. Rodenbrock A, et al. Development of a scalable fed-batch bioreactor process for high-titer production of lentiviral vector using an inducible HEK293 producer cell line. BioProcessing Journal 23: Open access, 2024.
2. Manceur AP, et al. Scalable Lentiviral Vector Production Using Stable HEK293SF Producer Cell Lines. Hum Gene Ther Methods 28(6): 330-339, 2017. PubMed: 28826344
3. Broussau S, et al. Inducible packaging cells for large-scale production of lentiviral vectors in serum-free suspension culture. Mol Ther 16(3): 500-507, 2008. PubMed: 18180776
4. Manceur A, et al. Large-scale production and purification of a lentiviral vector reference material. BioProcess J [In Press], 2025.