Rapid and sensitive detection of Replication-Competent Lentivirus (RCL) using digital PCR

Replication-competent lentivirus (RCL) presents a critical safety risk in lentiviral vector–based therapies and must be rigorously excluded during manufacturing. Accurate, rapid and standardized detection of RCLs is essential to ensure patient safety and regulatory compliance in cell and gene therapy manufacturing workflows.

The QIAcuity RCL Quant Kit enables sensitive, fast and accurate detection and quantification of vesicular stomatitis virus G glycoprotein (VSV-G) DNA in a sample using digital PCR technology. An internal PCR control is incorporated into the kit to reliably and consistently identify PCR inhibitors that may affect assay performance. This duplex assay ensures consistent detection performance even in challenging sample matrices. By combining speed, accuracy and ease of use on the QIAcuity Digital PCR System, it supports reliable RCL monitoring during manufacturing and product release.

Key performance advantages include:

- Reliable detection of RCL presence or absence based on the VSV-G DNA target
- High sensitivity down to 0.35 copies/µL with a broad dynamic range up to 7,500 copies/µL
- High linearity maintained even in complex samples containing up to 10 µg background DNA without prior dilution
- Rapid quantification of RCLs using digital PCR in under two hours with internal and positive controls
- Consistent and reproducible results supported by a simplified digital PCR workflow

Together, these features make the QIAcuity RCL Quant Kit a rapid and standardized solution for confirming the absence of RCL in lentiviral-based cell therapy manufacturing.

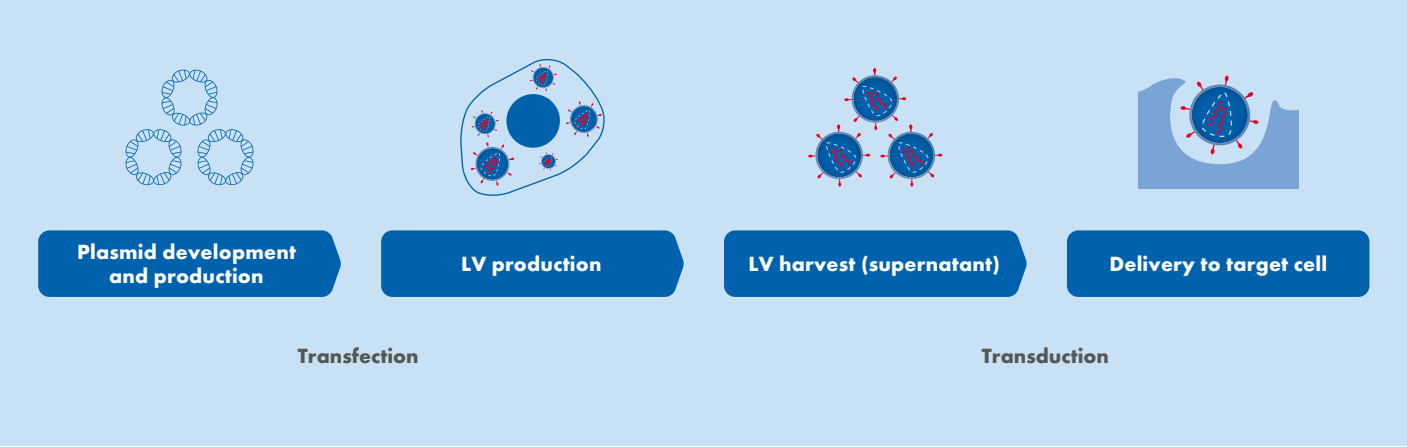


Figure 1. Schematic overview of the lentiviral vectors' transfection and transduction process. QIAcuity RCL Quant Kit is designed for highly precise detection of RCLs in target cells transduced with lentiviral vectors.

Standardization for reproducible and reliable RCL analytics

Standardized and reproducible RCL detection is essential for helping ensure patient safety and meeting regulatory expectations in lentiviral vector–based therapeutics. Lentivirus (LV) is a widely used viral vector in cell and gene therapy applications due to its ability to stably integrate genetic material into host cells. However, rare recombination events within plasmid sequences used to generate LVs can lead to the formation of RCLs, which can infect non-target cells and replicate, posing significant safety risks. To mitigate these risks, regulatory authorities mandate rigorous quality control testing to ensure the absence of RCLs throughout the manufacturing process (European Medicines Agency, 2020; U.S. Food and Drug Administration, 2020).

Accurate detection of RCLs is essential for the safety and efficacy of LV-based therapies. Assays must be robust and sensitive enough to detect even a single RCL event, providing confidence in the integrity of therapeutic products. Traditionally, the cell culture method is used to amplify potential RCLs in samples, but it may take up to 6 weeks for completion, which is impractical for the infusion of freshly transduced cells in many clinical trials. To address this challenge, the 2020 FDA guidance recognizes PCR-based methods as alternatives for rapid assessment of RCL in cell products intended for fresh release (U.S. Food and Drug Administration, 2020).

Digital PCR using the QIAcuity RCL Quant Kit provides unmatched sensitivity, specificity and accuracy, reducing time-to-result from weeks to hours. It offers a reliable and standardized method for confirming the absence of RCLs in ex vivo transduced cell batches, making it an ideal choice for rapid detection of RCLs.

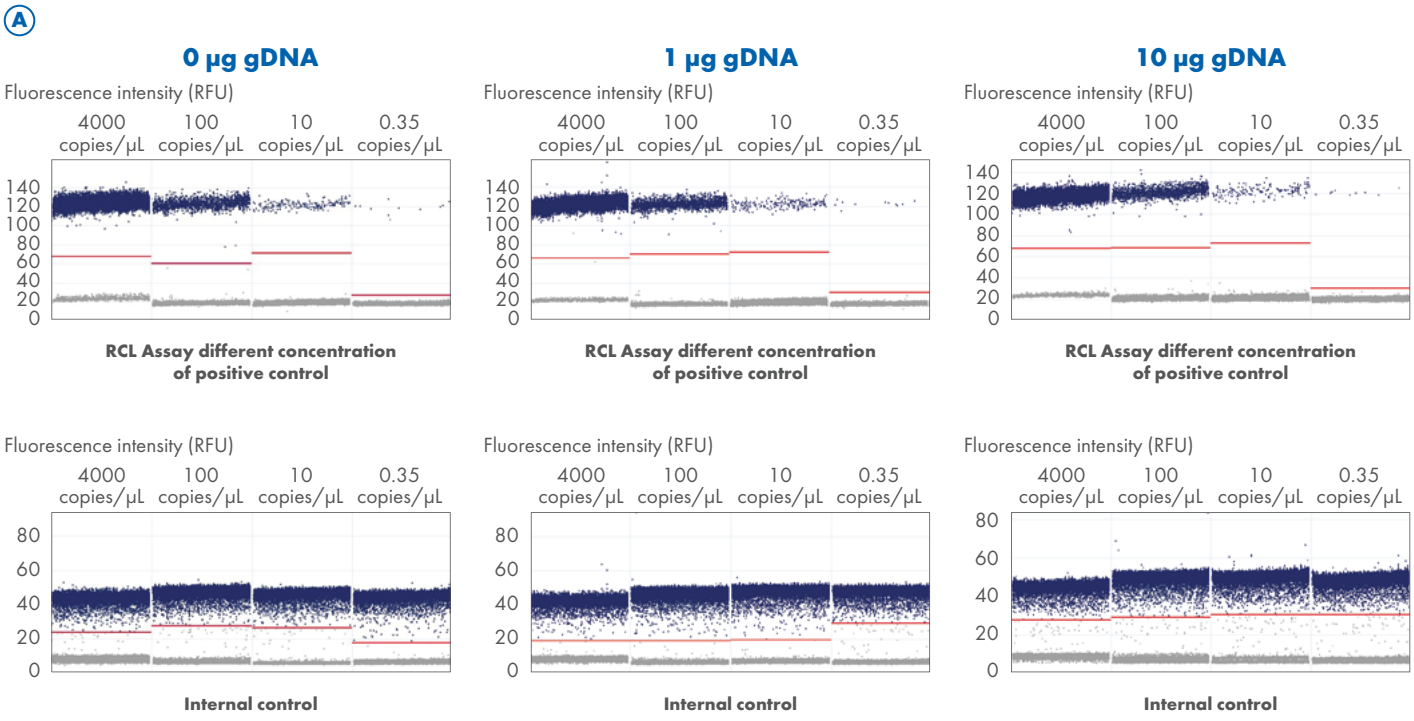
As a ready-to-use solution, the QIAcuity RCL Quant Kit enhances consistency across laboratories and manufacturing sites. Standardized reagents, protocols and controls reduce variability and support reliable data generation. This workflow promotes comparability of results across studies, simplifies regulatory review and strengthens confidence in the safety profile of lentiviral vector-based therapeutics.

High sensitivity and robust performance across complex sample matrices

Detecting RCLs can be challenging due to their potential presence at extremely low concentrations. Sensitive techniques like dPCR can identify RCLs at trace levels, helping to minimize the risks of inadvertent administration to patients. The QIAcuity RCL Quant Kit delivers exceptional analytical sensitivity and precision, ensuring consistent performance even at very low copy numbers.

The QIAcuity RCL Quant Kit can detect RCLs down to a low concentration of 0.35 copies/µL and maintains a high linearity across a broad dynamic range up to 7,500 copies/µL. Its robust performance is unaffected by high background genomic DNA (up to 10 µg per reaction), eliminating the need for prior dilution and ensuring reliable results even in complex sample matrices (Figure 2). This enables accurate quantification across various production stages, from upstream cell culture to final product testing.

These data highlight the kit's strong tolerance to non-target DNA and confirm its ability to deliver accurate, reproducible results under challenging conditions.



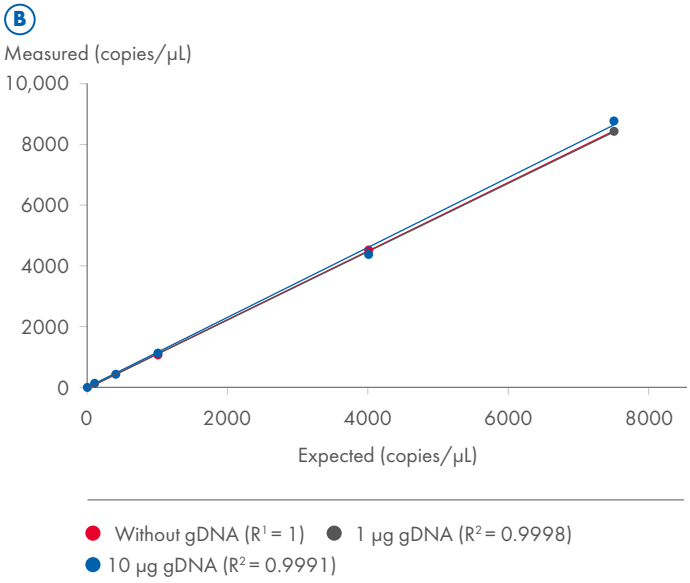


Figure 2. Increasing amounts of human background genomic DNA did not affect VSV-G detection using the QIAcuity RCL Quant Kit.
A 1 D Scatterplots of VSV-G Assay in FAM, QNIC (Internal Control) Assay in HEX. Different concentrations of VSV-G positive control (4000 cp/µL, 100 cp/µL, 10 cp/µL and 0.35 cp/µL) alongside an Internal Control (100 cp/µL) were spiked with increasing amounts of genomic background DNA (0 µg, 1 µg and 10 µg per reaction). dPCR was performed on a 24-well 26K nanoplate.
B The assay demonstrated high linearity and a broad dynamic range of 0.35–7,500 copies/µL, even in the presence of up to 10 µg of genomic DNA per reaction, without the need for prior dilution.

Summary of the technical specifications

To prepare the DNA, samples were extracted with the QIAamp DNA Mini Kit (QIAGEN) following the standard protocol. The RCL assay demonstrated a quantifiable range from 0.35 to 7,500 copies/µL. The Limit of Detection (LoD) was established at 0.35 copies/µL (95% Confidence Interval), defined as the lowest concentration at which at least 20 out of 21 replicates yielded a result above the Limit of Blank (LOB). The LoB was determined to be 0.00 copies/µL in all no-template controls (95% CI; 23 out of 23 replicates were negative). Assay robustness was evaluated by spiking reactions with up to 10 µg of human genomic DNA as background, with no adverse impact on detection performance, indicating high tolerance to non-target DNA (Table 1). ▷

These results confirm the reliability and reproducibility of the QIAcuity RCL Quant Kit for accurate detection and quantification of VSV-G DNA across a wide range of concentrations, even in complex sample matrices. Validated performance characteristics are summarized in Table 1.

The QIAcuity RCL Quant Kit combines high sensitivity, precision and reproducibility to deliver rapid, standardized RCL detection using digital PCR. Its validated workflow provides accurate quantification across a broad dynamic range and remains robust. By reducing time-to-result from weeks to hours and harmonizing methods across manufacturing sites, the kit supports safer, faster and more reliable release of lentiviral-based therapies.

Table 1. Technical specifications of the QIAcuity RCL Quant Kit

Parameter	Specification
Target	VSV-G DNA
Limit of Blank (LoB)	0.00 copies/µL
Limit of Detection (LoD)	0.35 copies/µL
Dynamic range	0.35–7,500 copies/µL
Specificity	No detection of human genomic DNA
Background DNA tolerance	Up to 10 µg per reaction

References

European Medicines Agency. (n.d.). *Guideline on the development and manufacture of lentiviral vectors.* Retrieved from https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-development-and-manufacture-lentiviral-vectors_en.pdf

U.S. Food and Drug Administration. (2020). *Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-Up: Guidance for Industry.* Retrieved from https://www.fda.gov/media/113790/download

Ordering Information

Product	Contents	Cat. no.
QIAcuity RCL Quant Kit	For 96 reactions: QIAcuity MasterMix, QN Internal Control DNA, QN IC Probe Assay, VSV-G Assay, Positive Control, RNase-free water	250322

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