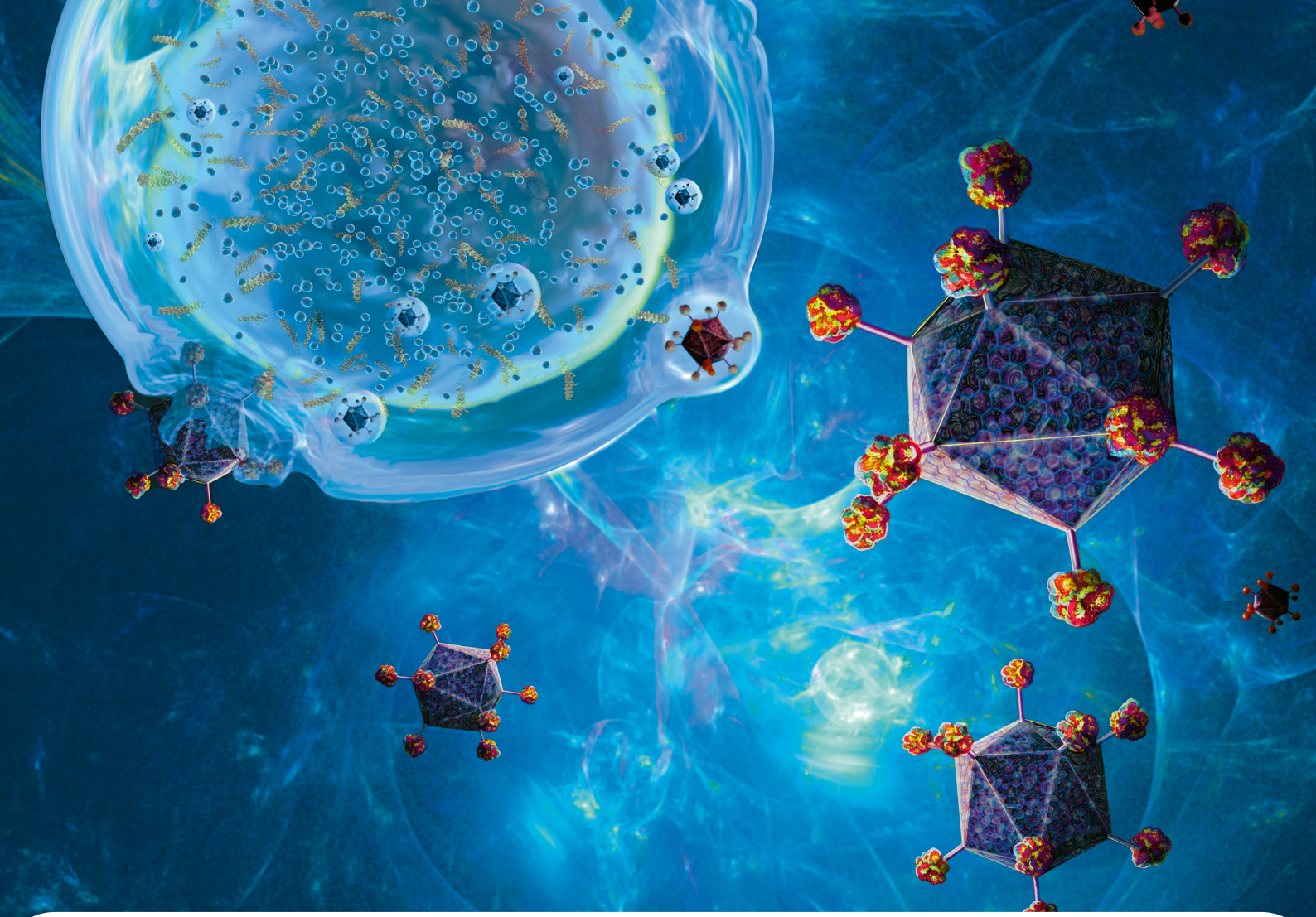


QIAGEN

Advancing cell and gene therapy development with digital PCR

Sample to Insight

Introduction

Cell and gene therapies seek to target diseases at their source, promising individualized treatment and possibilities for previously untreatable diseases. Ensuring the safety, potency and purity of therapeutic products at every stage of development is of high importance. From plasmid preparation to viral vector production and final product testing, rigorous quality control and innovative technologies are essential to bring these breakthroughs to life.

This brochure showcases cell and gene therapy instruments and solutions that empower researchers, manufacturers and quality control teams to overcome these challenges with confidence. Explore a portfolio containing digital PCR (dPCR), assays and advanced software — all designed to accelerate your path from discovery to commercialization.

With recent advances in dPCR, it is increasingly becoming the technology of choice for robust manufacturing and quality control processes. We took dPCR one step further with the QIAcuity Digital PCR System, a fully integrated system based on nanoplate technology that promises a faster time to result, higher throughput, multiplexing and scalability.

The system is coupled with feature-rich QIAcuity Software Suite 3.1, which enables labs to adhere to 21 CFR Part 11 compliance requirements in a GMP setting.
Whether you're pioneering new treatments or scaling production to meet global demand, our holistic solutions are here to help you achieve your vision of better, safer therapies—faster than ever before.

Contents

dPCR: key technology for QA/QC of cell and gene therapy

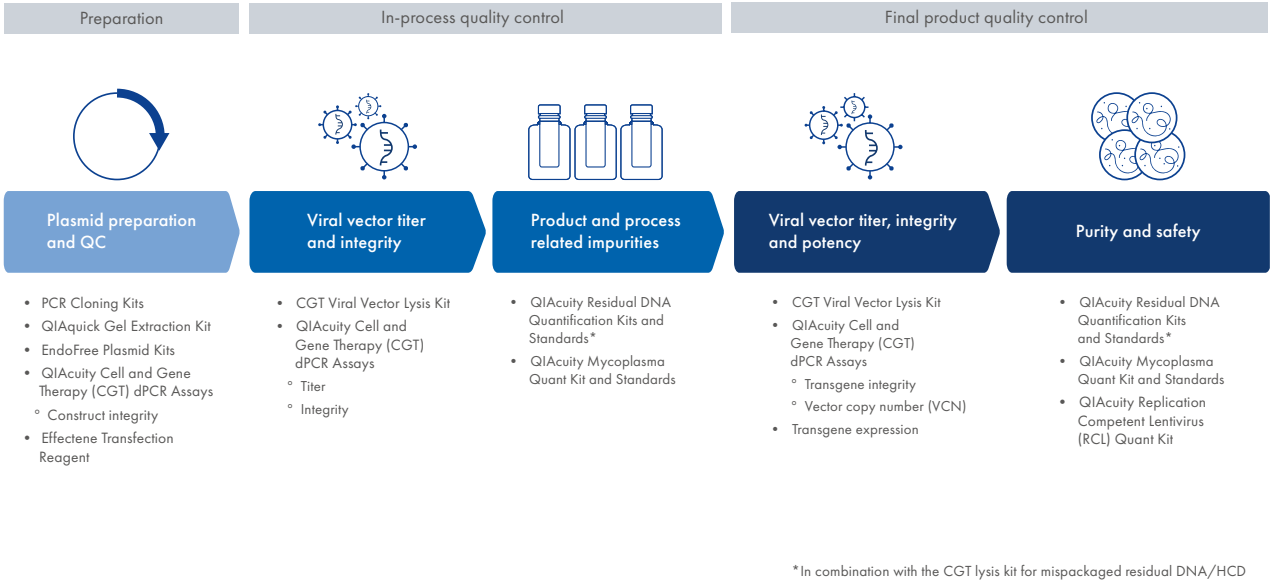
Cell and gene therapies are inherently complex, with a large degree of variability. The superior accuracy and precision offered by digital PCR enable you to ensure the quality and reproducibility of the research results through the use of your therapeutic product. Not only can you reliably and precisely determine viral vector quality, titer and integrity, but you can also perform robust contaminant testing by detecting mycoplasma and residual host cell DNA.

Gene therapy

Gene therapy involves the transfer of genetic material, usually in a carrier or vector, and the uptake of the gene into the right cells of the body. This can include inserting a new copy of a gene and gene editing using technologies such as CRISPR and gene silencing. It is usually conducted via viral-mediated gene transfer with adeno-associated viruses (AAVs) and lentiviruses (LVVs).

dPCR is an essential tool in gene therapy development in manufacturing environments, particularly for process development and quality control. It provides higher reproducibility and precision at a lower template input range, enabling more robust results.

Discover our assays and solutions in each step of gene therapy manufacturing in this workflow.



*In combination with the CGT lysis kit for mispackaged residual DNA/HCD

Cell therapy

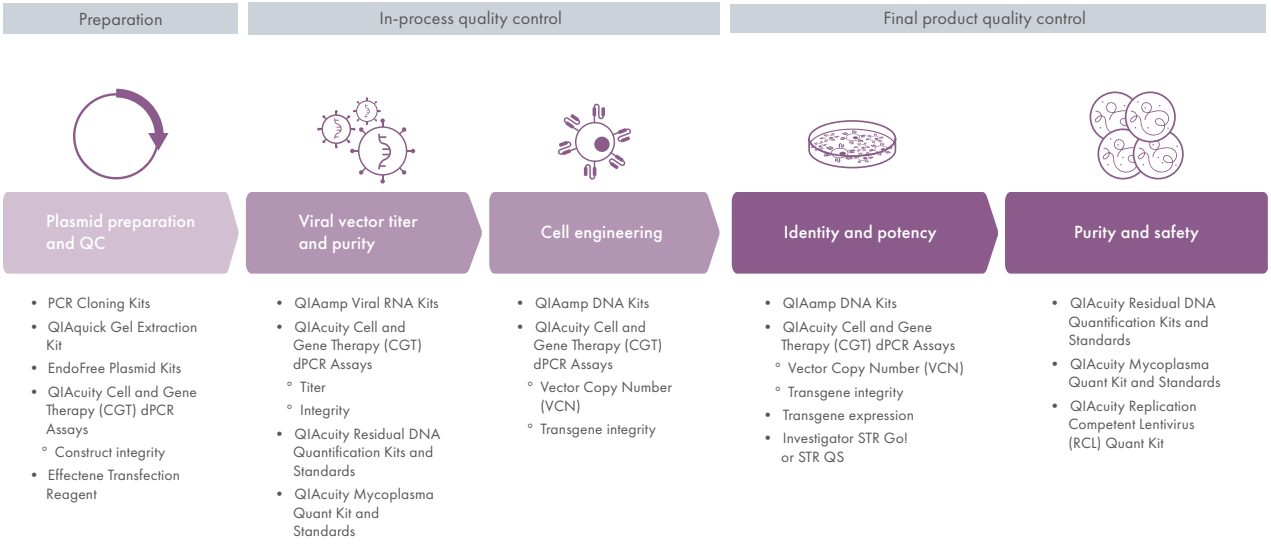
Cell therapy involves the transfer of functional cells that can be modified outside the body and are then injected into patients. These cells can be from the patient (autologous) or a donor (allogeneic).

dPCR improves the precise quantification of modifications in these therapeutic cells, leading to accurate control over gene editing events. It also helps detect contaminating DNA, guaranteeing its safety and purity and ensuring reproducibility in lentivirus for CAR-T cell therapy production. It is also essential for detecting minimal residual disease (MRD) in cancer therapy, scanning therapy-related biomarkers and providing quality control during manufacturing.

"We tested QIAGEN's QIAcuity dPCR for quantification of viral titer, vector copy number and residual host cell DNA – all critical to in-process quality control in gene therapy. It is easy to use, fast, scalable and complies with requirements for GMP. The system is a great addition to our analytical development and testing services, process development and R&D platforms which is available to our clients now."

Dana Cipriano, Senior Vice President, Testing and Analytical Services, Center for Breakthrough Medicines in King of Prussia, PA, in the U.S.

Learn more about where our kits, instruments and solutions can be integrated into cell therapy in this workflow.



Adeno-associated virus (AAV) characterization

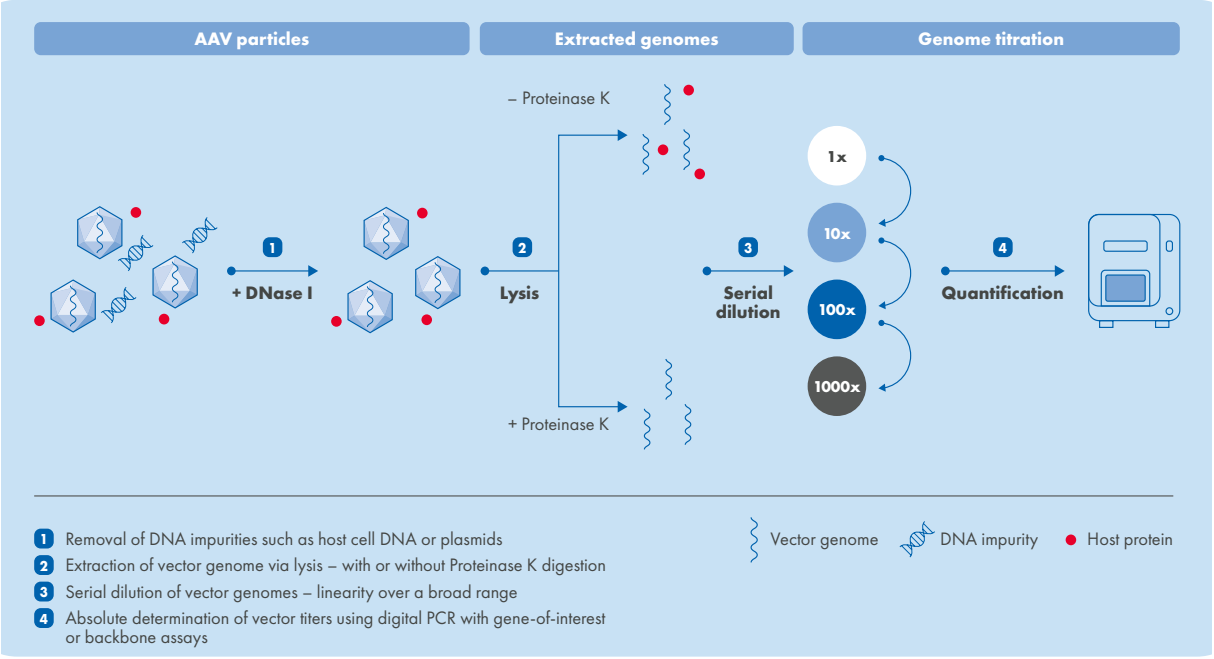
Adeno-associated virus (AAV) is a widely used viral vector in gene therapy applications. However, the generation and purification of the viral vectors require rigorous quality control to enable safe and reliable dosing during clinical studies or patient care. The ability to accurately characterize and quantify critical quality attributes (CQAs), such as vector titers and integrity, and detect contamination is crucial for safe and effective AAV-based gene therapies.

Standardized genome quantitation

We offer a standardized workflow from capsid lysis to quantification of the viral titer from cell lysates, using the CGT Viral Vector Lysis Kit with dedicated QIAcuity® Cell and Gene Therapy (CGT) dPCR Assays and QIAcuity Probe PCR Kit on the QIAcuity Digital PCR System. This workflow provides significant advantages in accuracy, reproducibility, speed and ease of use, enabling robust vector genome titration that is critical to manufacturing and determining clinically effective dosages for therapies.

Main features:

- Standardization of AAV lysis with more straightforward SOP implementation and QC of current viral titration workflows
- Consistent and reproducible measurement of viral titers for multiple serotypes
- Accurate quantification down to 0.3 copies/µL
- Robustness of <10% CV between operators and assays
- Ability to run single-plex or multiplex reactions
- Compatibility with both unpurified in-process and purified samples and with final-release testing samples



AAV titer determination workflow using the QIAGEN CGT Viral Vector Lysis Kit and CGT dPCR Assays: In a first step, AAV samples are treated with DNase I to remove DNA impurities. In step 2, DNase I-treated samples are lysed in the presence or absence of Proteinase K. In step 3, the lysates are serially diluted. Finally, in step 4, the viral titers and integrity status are determined using a QIAcuity dPCR instrument.

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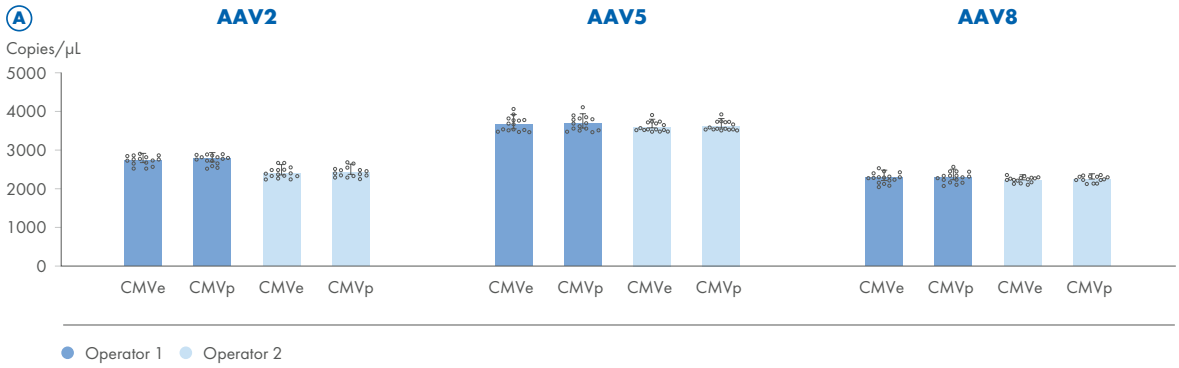

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Sample	Operator	Coefficient of variation between operators (%)		Coefficient of variation between replicates of one operator (%)	
		CMVe	CMVp	CMVe	CMVp
AAV2 (VB)	1	8.6	8.7	4.5	4.4
	2			5.7	5.3
AAV5 (VB)	1	1.2	1.2	5.1	5.0
	2			3.6	3.4
AAV8 (VB)	1	1.4	1.4	5.9	6.0
	2			3.1	3.2

High inter- and intra-assay precision and reproducible independent of operators, replicates and sample types.
Operator 1 represents an experienced dPCR user, whereas Operator 2 represents a first-time dPCR user.

Learn more about our QIAcuity Cell and Gene Therapy (CGT) dPCR Assays.

Vector genome integrity with digital PCR

The importance of determining genome integrity becomes particularly evident in the analysis of viral vectors such as AAVs, which are known for their susceptibility to packaging errors. The QIAcuity dPCR System with the QIAcuity Software Suite offers a powerful solution for determining DNA integrity and stability, and genome integrity determination benefits from higher multiplex capabilities. Moreover, dPCR is a valuable tool for assessing DNA stability, providing valuable insights into storage and processing impacts.

Read the application note on the detailed analysis of the integrity calculation.

Lentivirus (LV) characterization

Lentiviral vectors (LVVs) are powerful gene delivery tools widely used in cell and gene therapy due to their high packaging payload and ability to integrate into the host genome, enabling long-term expression.

Accurate vector characterization, including VCN and titer determination, and detecting replication competent lentiviruses (RCLs), are crucial for regulatory compliance, production consistency, and therapeutic success while minimizing the risks of insertional mutagenesis.

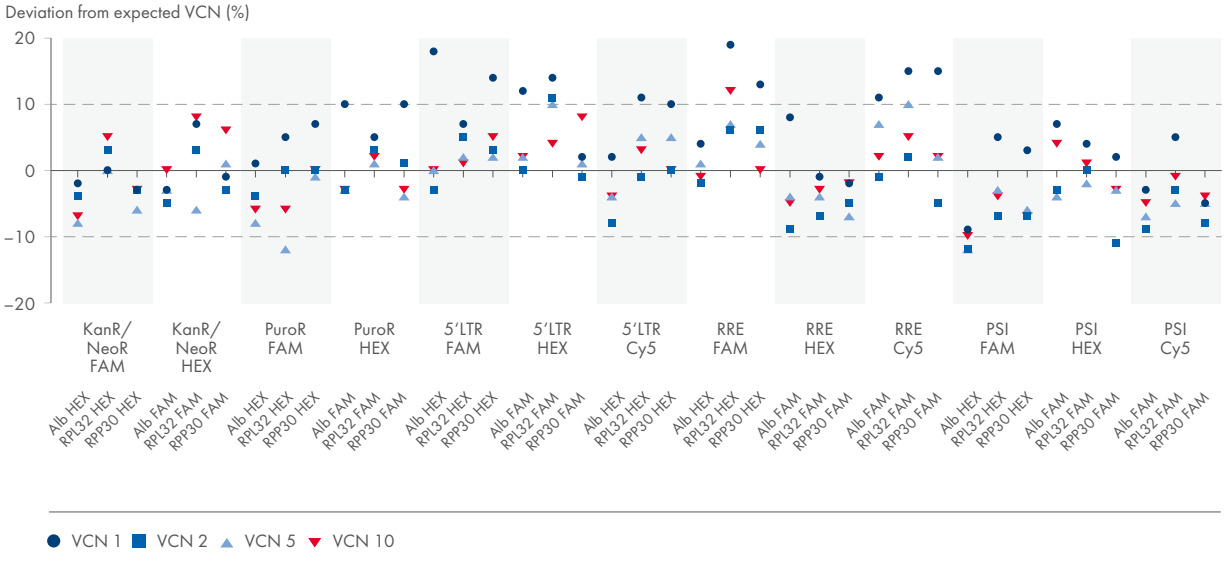
Absolute quantification of vector copy number (VCN) and viral titer

We offer a standardized workflow on the QIAcuity Digital PCR System with a mix-and-match approach using QIAcuity® Cell and Gene Therapy (CGT) dPCR Assays dedicated to lentiviral vectors. Choose from optimized vector backbone assays for integration plasmid elements, regulatory elements or reporter/ resistance genes, to quantify precisely LVV titer, or in combination with genomic reference assays to assess VCN easily.

Main features:

- Standardized LVV workflows for VCN and viral titer measurement
- High precision over a broad dynamic range (0.25 copies/µL – 7,500 copies/µL)
- Precise and comparable VCN determination of different LVV targets and reference genes from 0 to over 20 copies per genome
- Ability to run single-plex or multiplex reactions

Learn more about our QIAcuity CGT dPCR Assays.



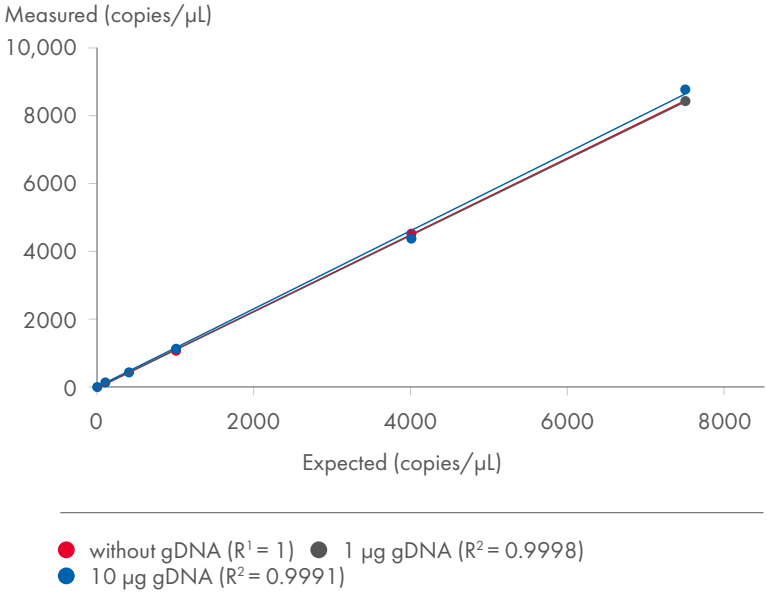
High accuracy for VCN quantification independent of fluorophores. CGT dPCR vector backbone assays (KanR/NeoR, PuroR, 5'LTR, RRE, Psi) run in duplex reaction with CGT dPCR genomic reference assays (Albumin, RPL32, RPL30). Template was gDNA and 0 / 1 / 2 / 5 /10 fold concentration of gBlocks. All assays had a deviation from expected VCN of less than 20%. For 24 out of 39 duplex reactions deviation from expected VCN is less than 10%. PCR was performed on a 96 8.5K nanoplate.

Replication-competent lentivirus (RCL) detection

During manufacturing, careful monitoring of viral vectors is essential to detect replication-competent lentivirus (RCL), which can pose serious safety risks, such as insertional mutagenesis and unintended infections. RCLs can arise from rare recombination events, necessitating strict regulatory compliance. Guidelines recommend RCL testing at multiple stages, but traditional cell culture-based methods are slow and labor-intensive.
As a faster, highly sensitive alternative, the QIAcuity RCL Quant Kit provides precise, robust detection of RCLs on the QIAcuity digital PCR System, streamlining quality control and accelerating CGT development.

Key features:

- Accurate results on the absence or presence of RCL based on the detection of envelope gene sequences VSV-G (vesicular stomatitis virus G glycoprotein)
- High sensitivity down to 0.35 copies/µL (Dynamic range of 0.35–7,500 copies/µL)
- High linearity even in the presence of up to 10 µg gDNA without the need for prior dilution
- Reproducible results and simplified dPCR workflow



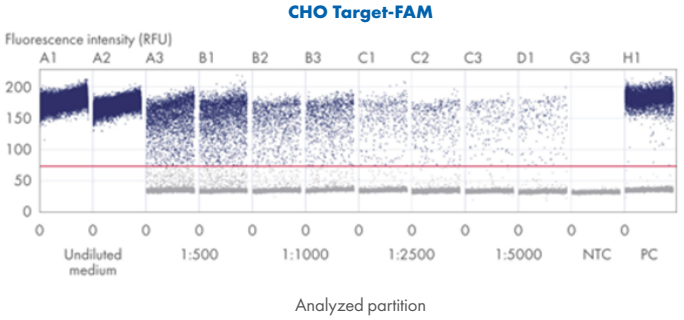
Linear and robust detection of RCLs using the QIAcuity RCL Quant Kit. VSV-G Assay in FAM, QNIC Assay in HEX. Template: gBlock 7500 / 4000 / 1000 / 400 / 100 / 10 / 0,35 copies/µL + QNIC 100 copies/µL with 0 / 1 / 10 µg gDNA background. PCR was performed on a 24 26K nanoplate. The coefficients of determination were 0.99.

Explore the benefits of QIAcuity RCL Quant Kit.

Residual host cell DNA (HCD) monitoring

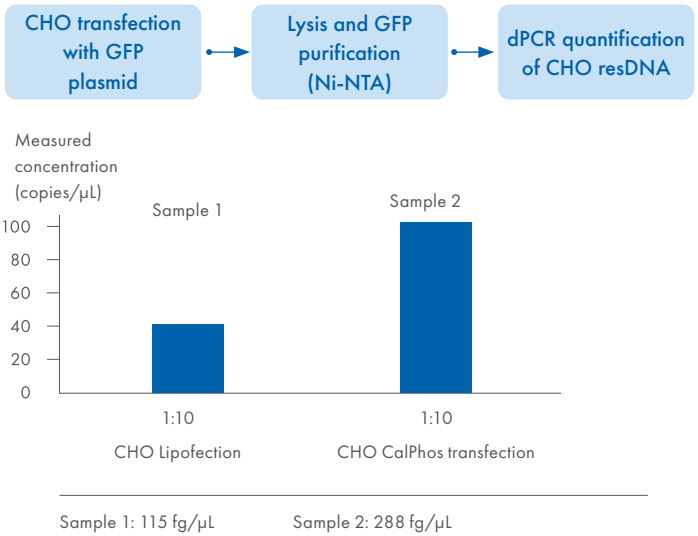
Residual host cell DNA (HCD) monitoring is an important step in the process of manufacturing proteins and vaccines, as the potential carryover of HCD poses a safety concern. Levels of HCD must not exceed those established by regulatory agencies such as the U.S. Food and Drug Administration and the World Health Organization. Digital PCR provides higher detection sensitivity at a lower template input range than qPCR, enabling a more robust application. The QIAcuity Residual DNA Quantification Kits provide accurate CHO, *E. coli* and HEK293 resDNA quantification results even in the presence of trace levels of PCR contaminants and other inhibitory reagents. Multicopy target assays ensure that results are unaffected by the fragmentation level of the resDNA.

Application 1: Residual DNA quantification without extraction



Sample dilution	Concentration (copies/µL)	CI (95%)
Undiluted	N.A.	N.A.
1:500	176.25	3.50
1:1000	61.89	5.80
1:2500	23.77	9.40
1:5000	12.15	13.05

Application 2: Residual DNA quantification from purified proteins



Residual host cell DNA can be detected from various samples.

Explore the quantification kits and their standards.

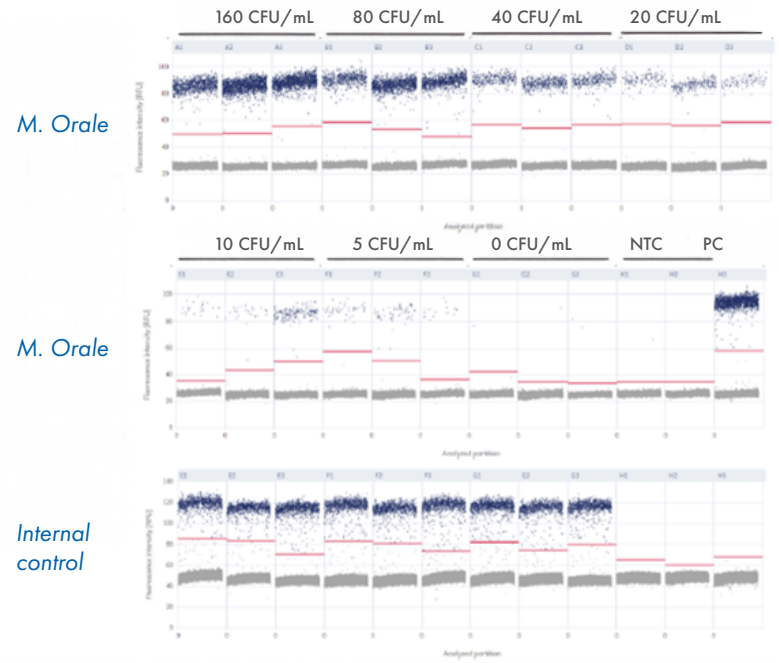
Mycoplasma detection

Mycoplasmas are common contaminants in biological products derived from cell lines in the biopharmaceutical industry. They are often introduced either from the source cell lines or during production. Digital PCR is an effective method for detecting these mycoplasma contaminations in cell cultures and related biological products.

The QIAcuity Mycoplasma Quant Kit is a highly sensitive RT-dPCR kit that detects rRNA and DNA, covering 127 mycoplasma species. The kit includes an internal control to prevent false negatives caused by PCR inhibitors or errors in RNA extraction and RT reactions.

QIAcuity Mycoplasma Quant Kit workflow is validated to meet pharmacopeia requirements

RT-dPCR 1D scatterplots of M. orale in DMEM +10% FCS and Internal Control Spike-In



Limit of detection of different Mollicutes species

Species/sample	Sensitivity
Acholeplasma laidlawii	10 CFU/mL
Mycoplasma arginini	5 CFU/mL
Mycoplasma fermentans	5 CFU/mL
Mycoplasma hyorhinis	10 CFU/mL
Mycoplasma orale	10 CFU/mL
Mycoplasma salivarium	5 CFU/mL

Species/sample	Sensitivity
Mycoplasma synoviae	10 CFU/mL
Spiroplasma citri	10 CFU/mL
WHO International Standard	10 IU/mL

Learn more about the product.

QIAcuity Software Suite and Services

Reliable and efficient GMP compliance support

QIAcuity Software Suite 3.1 is an integral part of the QIAcuity Digital PCR System, enabling users to set up plates, analyze results, and monitor runs in real time. It is designed to meet the stringent documentation requirements of 21 CFR Part 11 regulations.

Installation and Operational Qualification (IQ/OQ)

A service that provides information about the performance of all 5 channels of the QIAcuity platform for installation and regular service.

- Ensures instrument accuracy and precision
- Enables quick lab workflow integration

What can you expect?

- Advanced customizable user management
- Electronic signature for reports
- Audit trail and traceability
- Improved plate permissions
- Increased cybersecurity
- Improved image analysis algorithms

This offering consists of:

- Dedicated QIAcuity 5-Channel OQ Service
- QIAcuity 5-Channel OQ Kit

Unlock more resources about cell and gene therapy at:
www.qiagen.com/cgtcontenthub

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