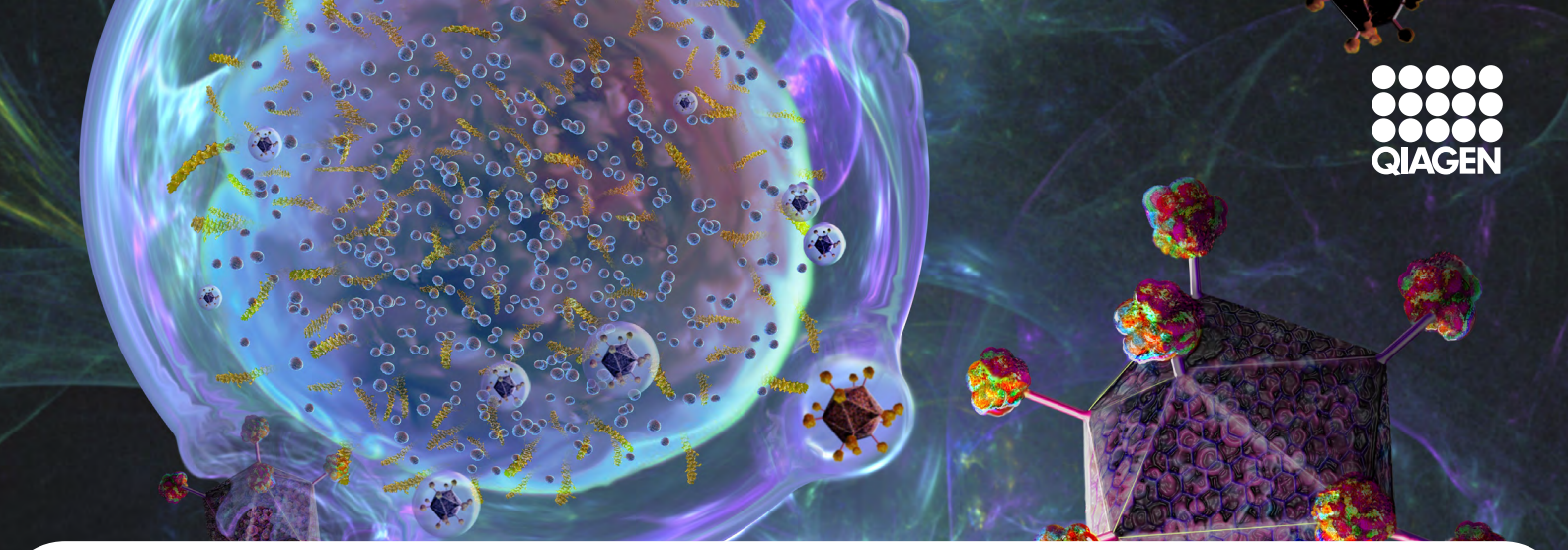
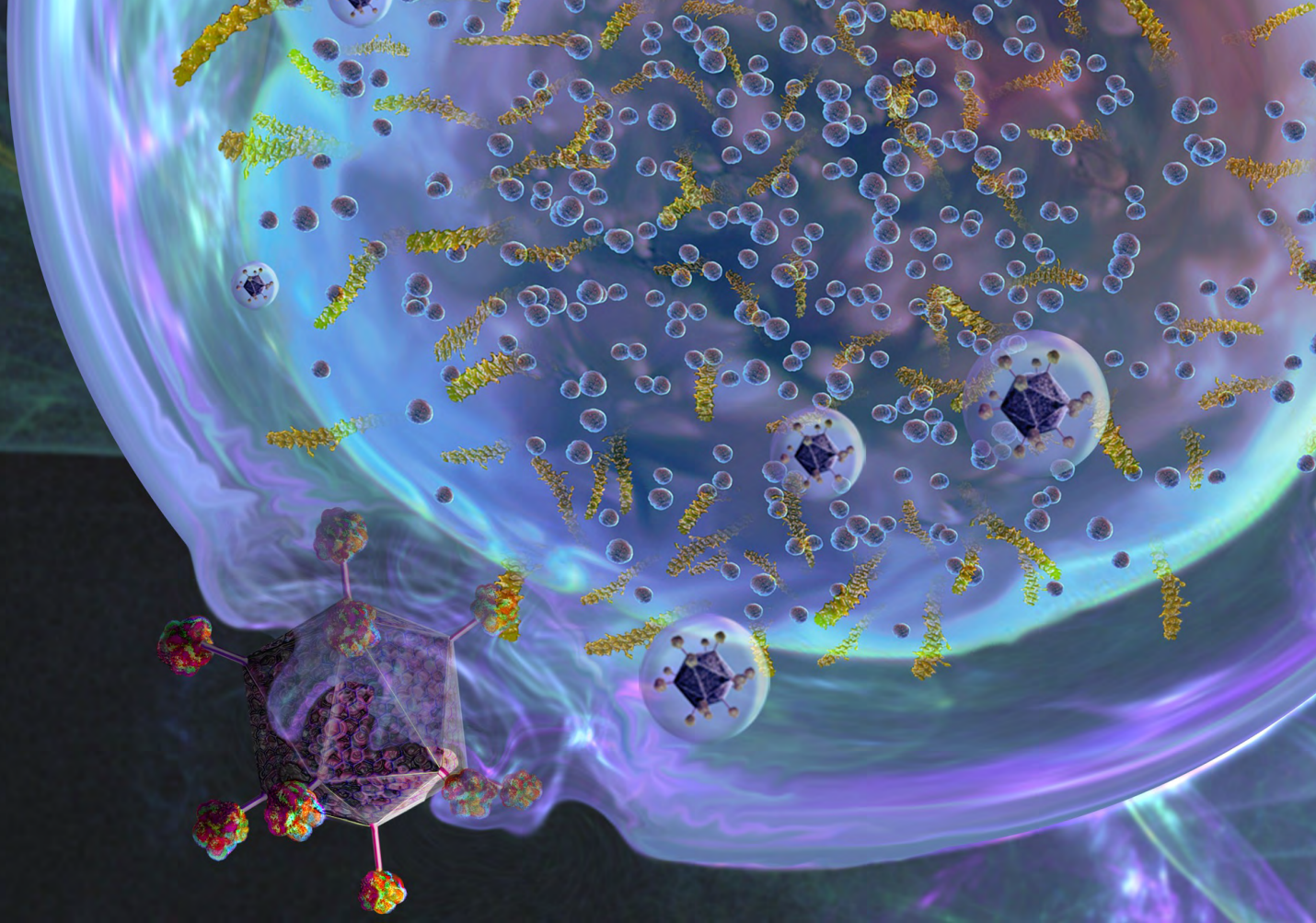
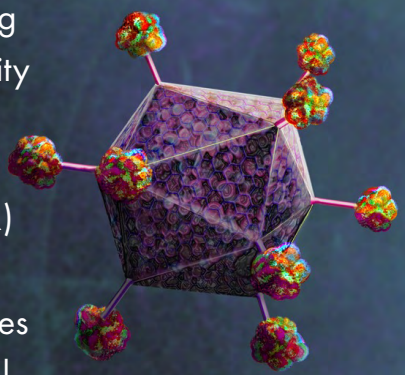


Successful cell and gene therapy development with QIAcuity® digital PCR

Sample to Insight



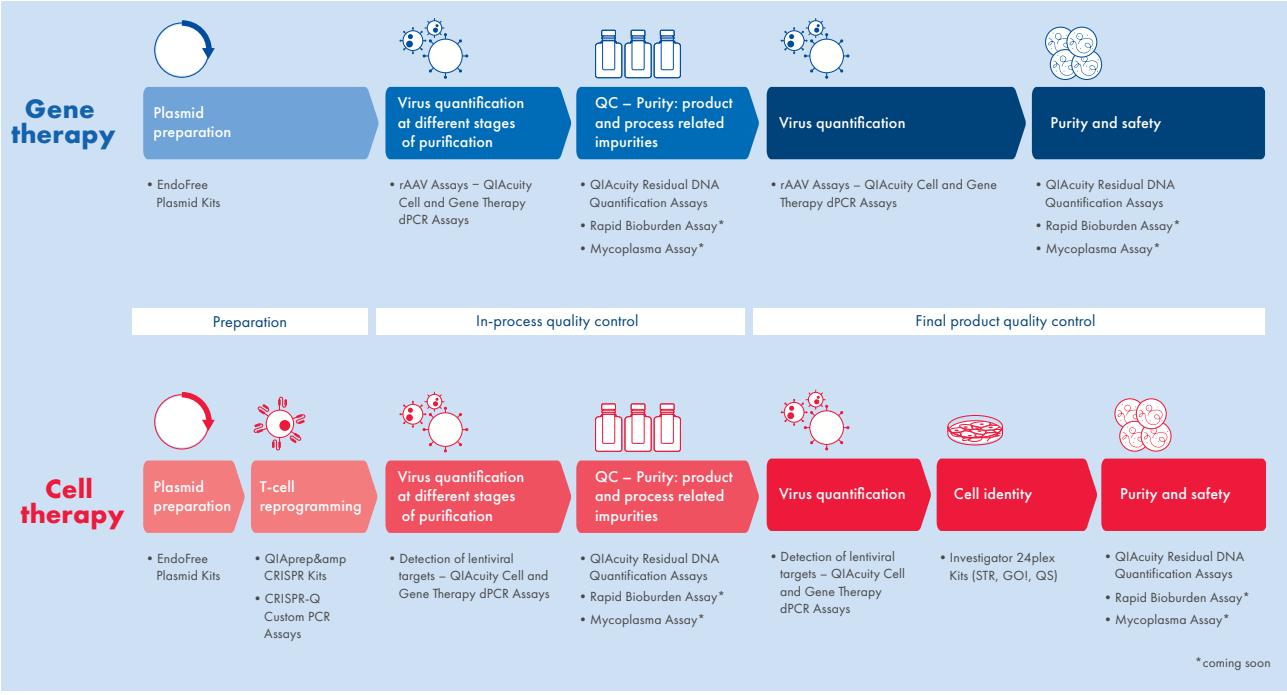
Cell and gene therapies seek to target diseases at their source, promising individualized treatment and possibilities for previously untreatable diseases.However, developing safe and effective cell and gene therapies requires quality control at all stages of the development process.



We provide fast, simple and scalable digital PCR (dPCR) workflow on the nanoplate-based QIAcuity system and connect you to high-quality assays, software and services to accelerate your journey from discovery to commercial-ization. Not only can you reliably and precisely determine plasmid quality and viral titer, but you can also perform robust contaminant testing for mycoplasma and residual host cell DNA. This ensures the potency, purity and safety of your therapeutic product.

Fully integrated assay portfolio for your cell and gene therapy applications

The assays integrate seamlessly with the QIAcuity instrument, nanoplate consumables, software and kits to offer the flexibility and accuracy required for your cell and gene therapy development and manufacturing applications.



> "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 at **https://www.qiagen.com/applications/pharma-biotech/applications/cell-and-gene-therapy**

Wet-lab tested kits and assays for the QIAcuity Digital PCR System

QIAcuity Cell and Gene Therapy (CGT) dPCR Assays

- Ten novel assay designs for individual targets (e.g., backbone targets)
- Double-quenched probes for best performance and background reduction
- Can be used in both singleplex and multiplex reactions. Can be additionally combined with gene-of-interest assays for absolute quantification of vector titer and assay robustness.

QIAcuity Residual DNA Quantification Kits

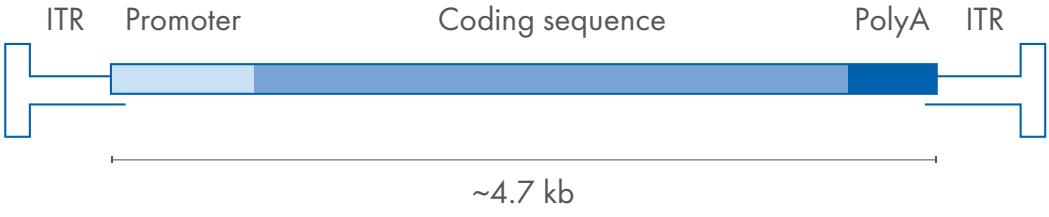
- Predesigned assays for CHO, *E. coli* and HEK293 host cell DNA (HCD) monitoring
- Multicopy target assays unaffected by the fragmentation level of the residual DNA (resDNA)
- Simple and fast extraction-free workflow for dPCR use

QIAcuity Cell and Gene Therapy (CGT) dPCR Assays

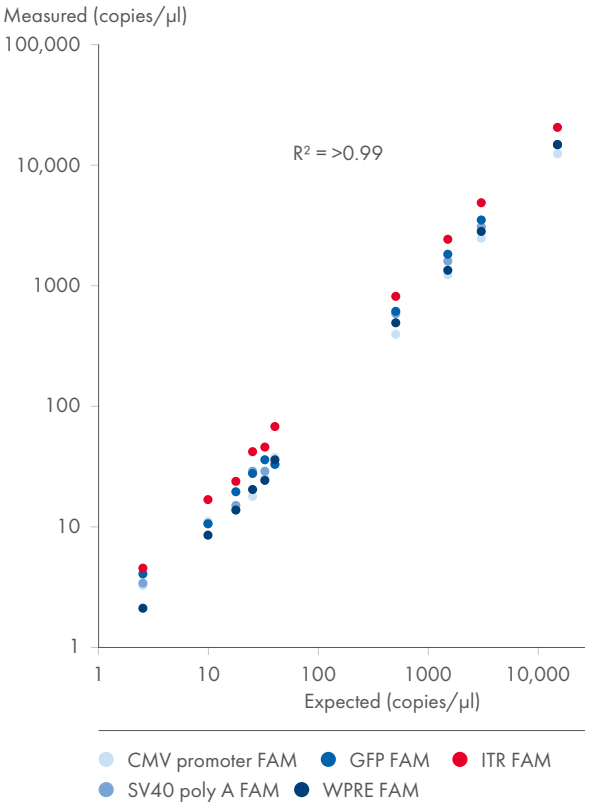
Accurate and precise AAV titer quantification

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 quantify vector titers and detect contamination is critical for safe and effective AAV-based gene therapies.

qPCR is a widely used method for AAV quantitation due to its sensitivity and ease of use. Nevertheless, well-characterized DNA standards and assays are needed for accurate quantification. With the QIAcuity Digital PCR System and its dedicated QIAcuity Cell and Gene Therapy dPCR Assays, vector genome titration can be achieved with outstanding accuracy, reproducibility and speed using a qPCR-like workflow. All in a single tube.

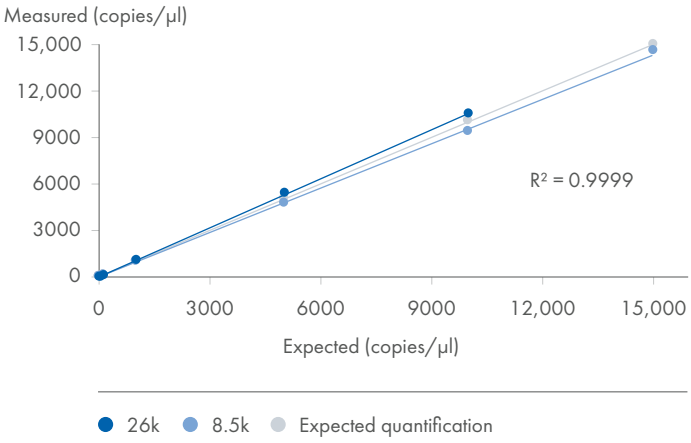


The Dependoparvovirus (AAV group) has a 4.7 kb ssDNA genome flanked by two ITRs with variable tropism. As a result of the transfer of the transgene via the AAV genome, a functional effect is achieved. Vector genome titers can be measured by PCR.



High precision over a broad dynamic range

The CGT dPCR FAM Assays were run on a serial dilution of DNA extracted from AAV2 samples. The DNA input ranged from 2.5–15,000 copies/µl. PCR was performed on an 8.5k Nanoplate. The coefficients of determination were >0.99. All assays had coefficients of variation less than 4%.



High precision over a broad dynamic range

The CGT dPCR SV40pA FAM Assay was run on a serial dilution of AAV DNA. The DNA input ranged from 2.5–15,000 copies/µl. PCR was performed on an 8.5k and a 26k Nanoplate. The grey line represents the expected quantification (linear scale). The coefficients of determination were >0.99. The coefficient of variation was less than 9% for the quantification on an 8.5k Nanoplate and less than 5% on a 26k Nanoplate. Experiments were run using at least 5 replicates per data point.

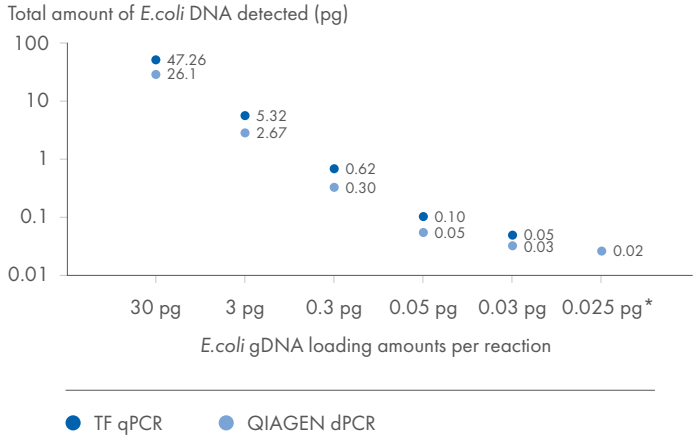
Visit **www.qiagen.com/qiacuity-cell-and-gene-therapy-dpcr-assays** to learn more

QIAcuity Residual DNA Quantification Kits

Precise and sensitive contaminant testing

Residual host cell DNA monitoring is an important step in the process of manufacturing proteins and vaccines. 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 sensitivity of detection at a lower template input range in comparison to qPCR and therefore enables 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. For example, QIAGEN offers the Certal Residual DNA Detection Kits, for qPCR-based detection of residual CHO host cell DNA. The QIAcuity CHO resDNA Quant Kit offers a more robust detection down to 5 fg (50,000–5 fg) compared to the Certal CHO Detection Kit which has a dynamic range of 10–300 ng.

Comparison – digital PCR vs. qPCR



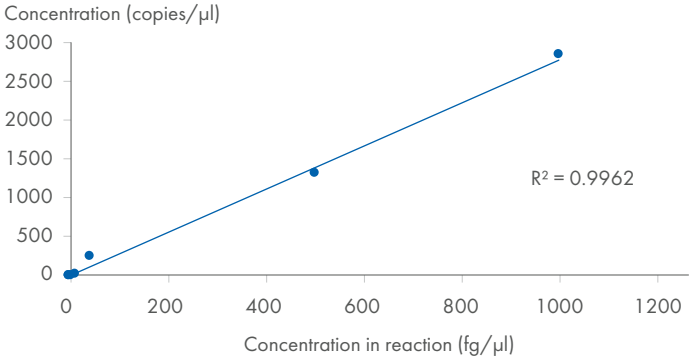
QIAcuity *E.coli* ResDNA Quant Kit (dPCR) provides higher accuracy at low-loading amounts
Reactions were set up according to the manufacturer's protocol. Results represent averaged values from duplicates.

*Below dynamic range of TF RNASEQ *E.coli* Quant Kit (qPCR)

Sample name	Template [pg/rxn]	Ct	Ct mean	Ct SD
QIAGEN CHO DNA	0.003	34.045	33.951	0.133
	0.003	33.857		
	0.03	30.769	30.691	0.110
	0.03	30.614		
	0.3	27.475	27.555	0.114
	0.3	27.636		
	3	24.110	24.144	0.048
	3	24.178		
	30	20.696	20.751	0.078
	30	20.806		
Thermo Fisher CHO DNA	0.003	34.738	34.511	0.321
	0.003	34.284		
	0.03	31.262	31.280	0.026
	0.03	31.299		
	0.3	28.265	28.303	0.055
	0.3	28.342		
	3	24.861	24.884	0.032
	3	24.907		
	30	21.430	21.476	0.064
	30	21.521		

Comparison of QIAGEN Standard vs. Thermo Fisher Standard for CHO on qPCR
The qPCR was done in duplicates. Ct mean and STDEV are calculated from duplicates.

Working range of QIAcuity CHO resDNA Quant Kit

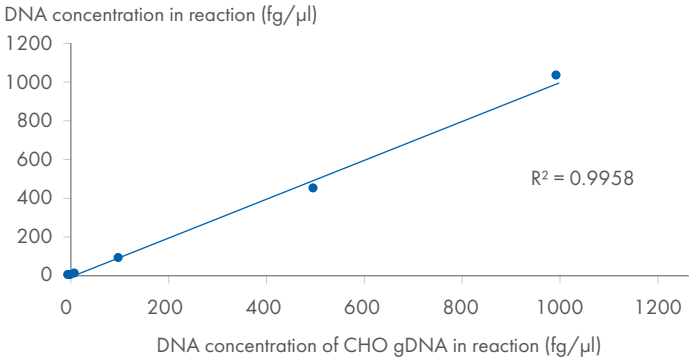


The working range performed with n=12 distributed in 4 runs with each dilution in triplicates

DNA target (fg)	DNA concentration in reaction (fg/µl)	Mean value (Copies/µl)	STDEV (Copies/µl)	Coefficient of variation (%)
40000	1000	2851.67	105.24	3.69
20000	500	1329.02	39.75	2.99
4000	40	257.83	9.84	3.82
400	10	26.48	1.21	4.58
40	1	2.53	0.14	5.71
10	0.25	0.69	0.12	16.78
4	0.1	0.32	0.09	28.07

A coefficient of variation ≤ 20% was obtained down to 40 fg input confirming a working range of 4000–40 fg DNA input.

Expected input DNA vs. measured CHO gDNA

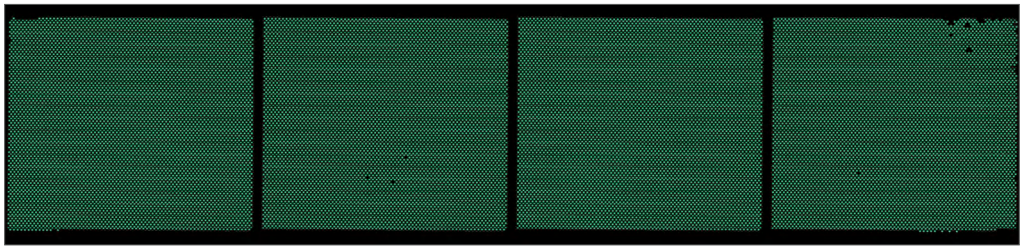


The expected amount of input DNA (fg/µl) versus the actual measured CHO genomic DNA shows R^2=0.9958. The data shown is the mean for n=3.

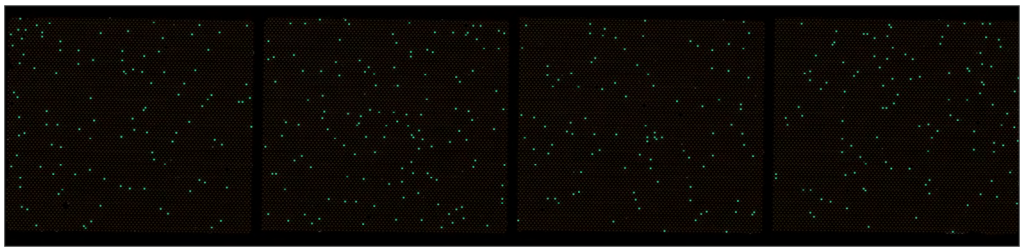
ResDNA testing in CHO cell culture medium

Residual DNA Quant Kits can detect residual host cell DNA from various samples, including cell culture media.

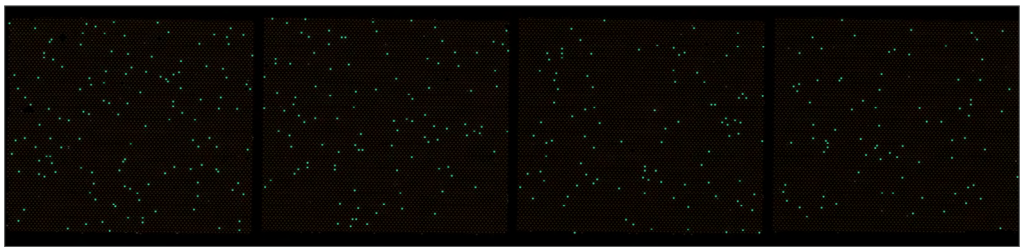
CHO cell medium



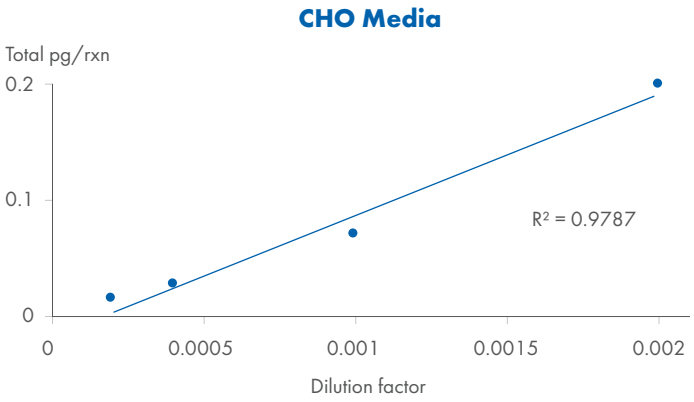
CHO cell medium, 1:2500 dilution, 0.27 copies/µl



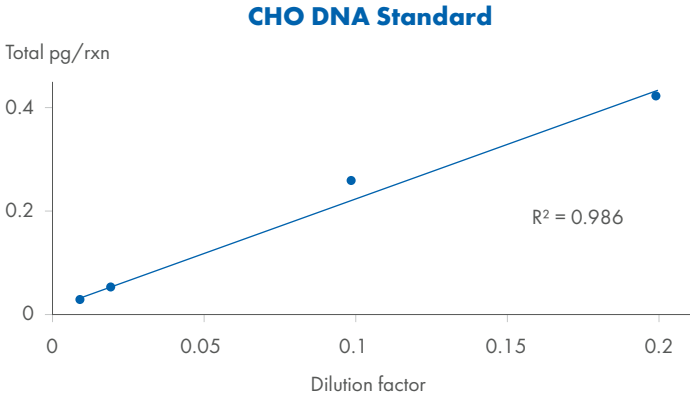
0.5 pg CHO Standard DNA, 0.26 copies/µl



Dilution series of medium from cultured CHO cells. TThe test was performed in compliance with the standard CHO ResDNA Quant Kit protocol. The test was performed in duplicate using QIAcuity Nanoplate 26k 24-well.



Data showing accurate detection of host cell DNA of CHO cells in cultured CHO medium down to 0.27 copies/µl equal to 0.5 pg input compared to CHO DNA Standard (below)

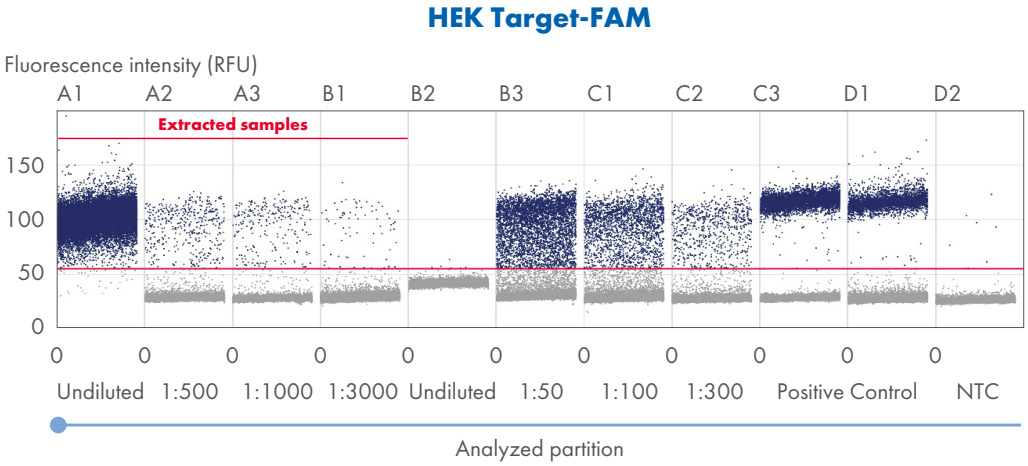


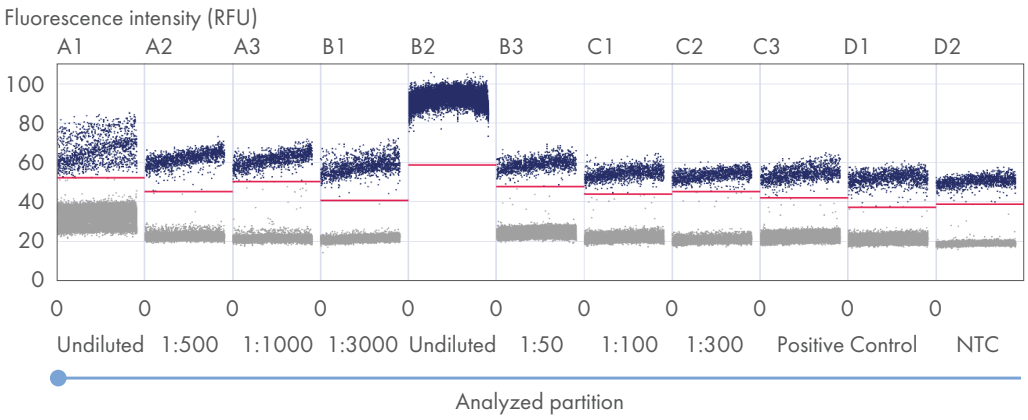
Data showing accurate detection of 0.5 pg (0.26 copies/µl) input of CHO DNA Standard

Visit **www.qiagen.com/qiacuity-residual-dna-quantification-kits** to learn more

Detection of residual DNA in AAV samples

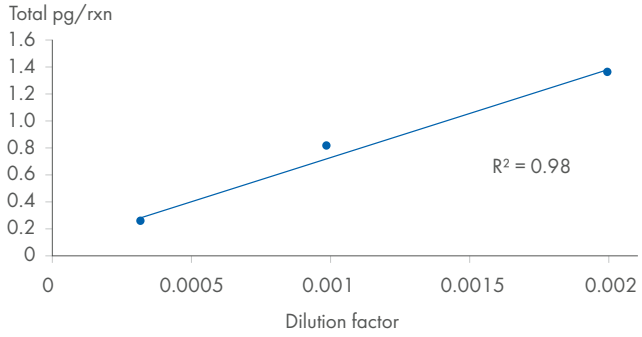
The ResDNA Quant HEK293 Kit provides a digestion- and purification-free workflow for the quantification of residual host cell DNA in AAV samples.





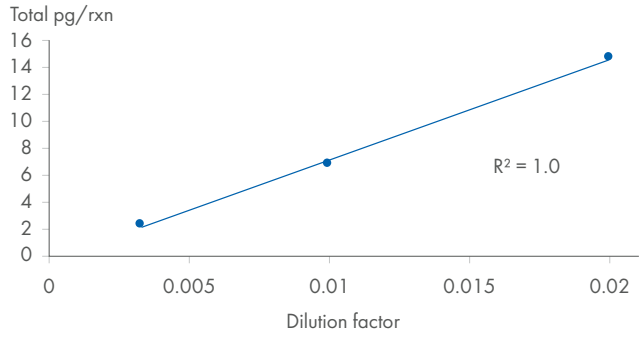
Host cell DNA was checked in cultured media for AAV2 produced in HEK293.
Samples were tested on the QIAcuity comparing extracted vs. unextracted host cell DNA. The extraction was performed with QIAsymphony Certal Kits according to the standard protocol. Standard QIAcuity HEK293 ResDNA Quant Kit protocol was used to detect residual DNA in the samples in a single well using QIAcuity Nanoplate 26k 24-well. A1-B1 shows extracted samples with NTC in B2. Unextracted samples are shown in B3-D2 with NTC. B2 for the internal control was overloaded, hence the concentration could not be calculated.

With extraction



Host cell DNA was checked in cultured media for AAV2 produced in HEK293.
In the dilutions of 1:500, 1:1000 and 1:3000 with a linearity of R^2=0.98.

Without extraction



Host cell DNA was checked in cultured media for AAV2 produced in HEK293.
In the dilutions of 1:50, 1:100 and 1:300 with a linearity of R^2=1.00.

Sample	Reaction mix	Target	Concentration (Copies/µl)
Undiluted	+extraction	HEK293	8635.6
1:500	+extraction	HEK293	21.92
1:1000	+extraction	HEK293	13.05
1:3000	+extraction	HEK293	3.89
Undiluted	-extraction	HEK293	0.323
1:50	-extraction	HEK293	238.7
1:100	-extraction	HEK293	112
1:300	-extraction	HEK293	37.95
PC	-	HEK293	314
PC	-	HEK293	176.6
NTC	-	HEK293	0.407
Undiluted	+extraction	IC	75.27
1:500	+extraction	IC	80.81
1:1000	+extraction	IC	80.03
1:3000	+extraction	IC	81.88
Undiluted	-extraction	IC	0
1:50	-extraction	IC	78.25
1:100	-extraction	IC	81.7
1:300	-extraction	IC	78.25
PC	-	IC	81.13
PC	-	IC	80.5
NTC	-	IC	78.26

The concentration of host cell DNA was measured in cultured media for AAV2 produced in HEK293, unextracted vs. extracted DNA with internal loading control (IC).

QIAcuity Software Suite and Services

Reliable and efficient with GMP compliance support

QIAcuity Software Suite 2.1 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.

What can you expect?

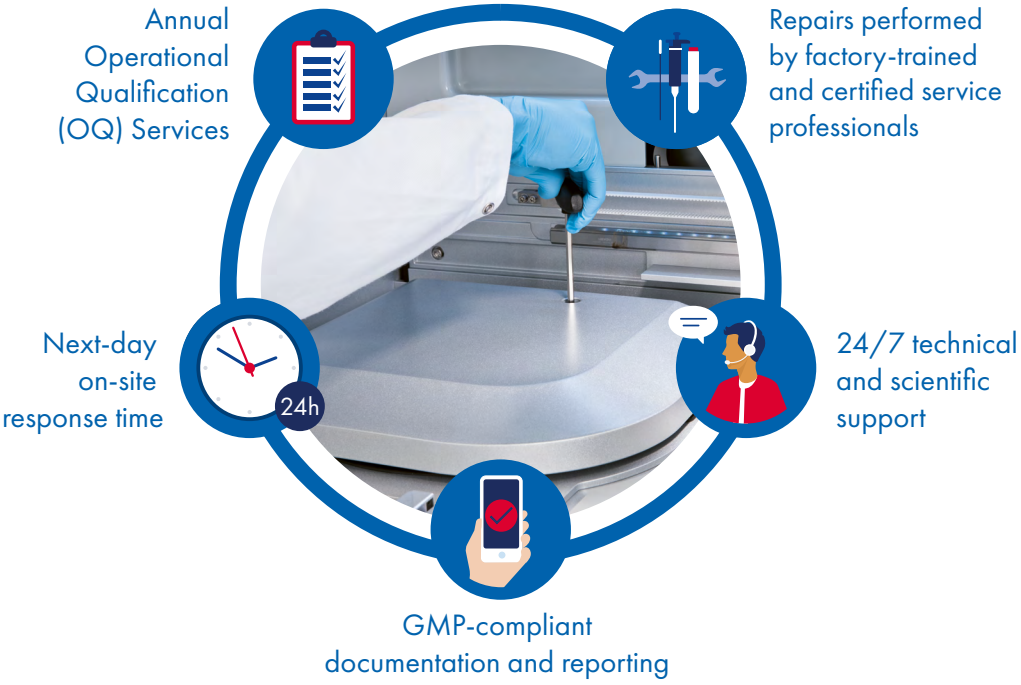
- **Advanced customizable user management**
- **Electronic signature for reports**
- **Audit trail and traceability**
- **Improved plate permissions**
- **Increased cybersecurity**
- **Improved image analysis algorithms**

Visit **www.qiagen.com/qiacuity-digital-pcr-system** to learn more

When working in a fast-paced manufacturing and QC environment, you must maximize the uptime and productivity of your instrument, reduce the risk of non-compliance, minimize disruption to your workflow and optimize performance. If something goes wrong, you should receive priority support and service.

Let us handle all the practical details and offer on-demand solutions to support your daily operations.

Enabling labs to adhere to 21 CFR Part 11 compliance requirements in a GMP setting



We can help:

- Optimize staff productivity
- Maximize instrument uptime
- Achieve cost control
- Limit quality risk
- Uphold GMP standards
- Strengthen audit documentation

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References:

1. A duplex assay for quantification and qualification of adeno-associated virus (AAV) using the QIAcuity Digital PCR System LINK
2. Optimized in-process recombinant adeno-associated virus (rAAV) vector genome titer protocol using the QIAcuity Digital PCR System LINK
3. Detection of vector copy number in bicistronic CD19xCD22 CAR T Cell products with digital PCR LINK
4. Digital polymerase chain reaction strategies for accurate and precise detection of vector copy number in chimeric antigen receptor T-cell products LINK

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To understand what a complete QIAcuity digital PCR cell and gene therapy solution could look like for you, please contact your local QIAGEN representative.

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