

The way forward in digital PCR

ViralSEQ dPCR Lentivirus Proviral DNA Titer Kit

Viral quantitation

Highly sensitive dPCR assay for quantitation of lentiviral proviral DNA in transduced cells

With the Applied Biosystems™ ViralSEQ™ dPCR Lentivirus Proviral DNA Titer Kit, proviral copy number can be used to calculate infectious lentiviral titers (as transducing units per mL (TU/mL)), and subsequently, the volume of recombinant lentivirus needed to transduce cells at the desired multiplicity of infection (MOI). The assay can also be used to determine vector copy number, a critical quality attribute (CQA) in cell and gene-modified cell therapy development. The assay targets a conserved region in the lentivirus genome and is designed to be compatible with most lentiviral transfer plasmids. The ViralSEQ dPCR Lentivirus Proviral DNA Titer Kit enables a wide dynamic range and high sensitivity for accurate detection of integrated provirus copies (Figures 1–2).

- Accurate quantitation of integrated genome copies by targeting a conserved region in the lentivirus genome
- Assay design compatibility with more than 200 lentiviral transfer plasmids
- Easy-to-use, integrated sample-to-results system features Applied Biosystems™ TaqMan™ digital PCR master mix and TaqMan™ primer/probe set


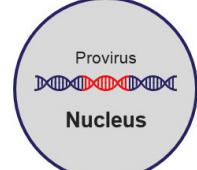


- Highly sensitive quantitation delivers results in about 3 hours
- Optional manual or automated sample preparation, optimized for quantitative recovery from bioproduction samples

The high sensitivity and specificity provided by the ViralSEQ dPCR Lentivirus Proviral DNA Titer Kit make it suitable for proviral quantitation in process development and lot release testing (Table 1).

Table 1

Specifications	
Linearity	$R^2 \geq 0.99$
PCR efficiency	$100\% \pm 10\%$
Precision	<20%
Limit of detection (LOD)	9 copies per reaction
Limit of quantitation (LOQ)	27 copies / rxn
Assay range	9 to 90,000 copies / rxn

The ViralSEQ dPCR Lentivirus Proviral DNA Titer kit can be used in conjunction with ViralSEQ dPCR Lentivirus Physical Titer Kit to correlate infectious and total viral particles by dPCR.

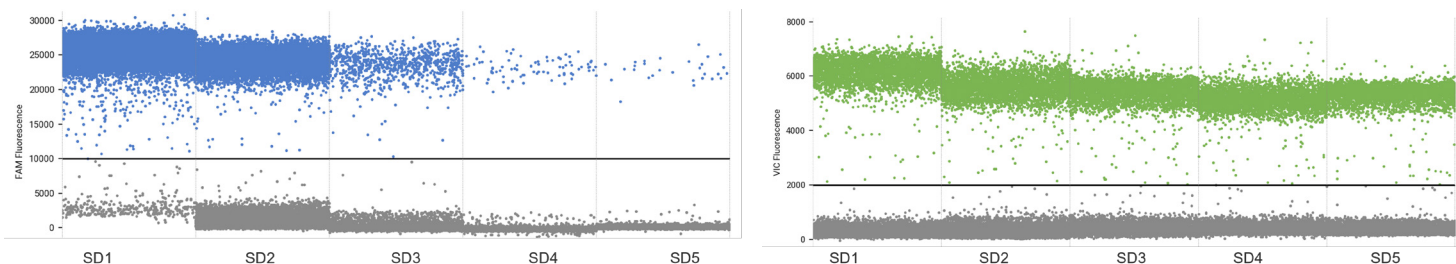


Figure 1. 1D dot plots displaying dPCR-based quantification of genome copies from serially diluted samples. Using the QuantStudio Absolute Q Digital PCR System, absolute quantification of viral copies across serially diluted samples is possible by counting the total number of microchambers positive for the fluorescent label.

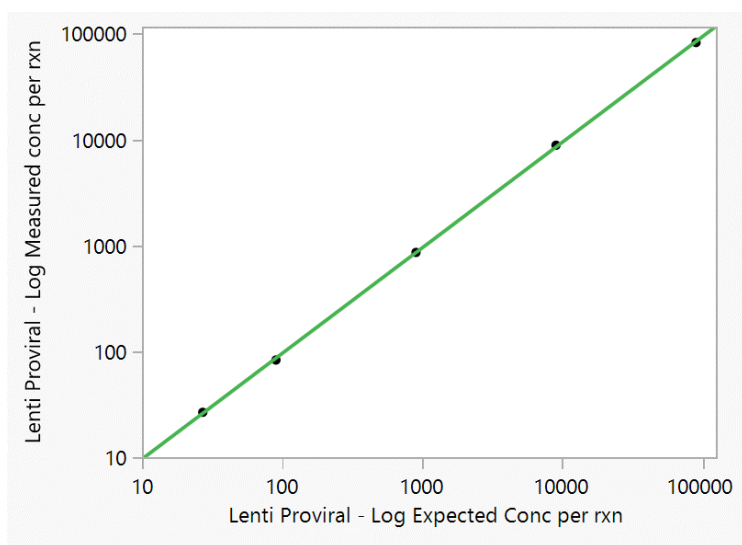


Figure 2. High sensitivity and broad dynamic range. Linear range was generated using serial dilutions ranging from 10,000 copies/ μ L (cp/ μ L) (SD1) to 3 copies/ μ L (SD5) of lentiviral particles. Linearity: $R^2 > 0.9994$ with an efficiency of 103.52%.

Table 2. ViralSEQ dPCR Lentivirus Proviral DNA Titer Kit performance summary demonstrates assay accuracy and precision among triplicate reactions. The copy number output can be converted to mass with a conversion factor.

<i>Lentiproviral</i>	Expected Conc. (cp/ μ L)	Inter-plate Precision			
		Ave. Conc. (cp/ μ L)	Relative Accuracy (%)	SD	CV%
SC1(ULOQ)	10,000.00	9586.6	95.87	194.10	2.02%
SC3	100.00	92.8	92.84	1.89	2.04%
SC4	10.00	9.5	95.20	0.36	3.80%
SC5(LOQ)	3.00	2.7	90.33	0.38	14.02%

Table 3. Level of detection (LOD)

	Expected Conc. of LOD (cp/ μ L)	Ave. Conc. of LOD (cp/ μ L)	Ave. Conc. of NTC (cp/ μ L)	SD of NTC (cp/ μ L)	Conc. of NTC + 3 SD (cp/ μ L)
LOD	1.00	0.95	0.05	0.11	0.37

Digital PCR workflow

The ViralSEQ dPCR Lentivirus Proviral DNA Titer Kit is part of an integrated digital PCR workflow for viral vector characterization during biopharmaceutical manufacturing (Figure 3). Optional use of the Thermo Scientific™ Pharma KingFisher™ Apex 96 Deep-Well Magnetic Particle Processor with the Applied Biosystems™ PrepSEQ™ Nucleic Acid Sample Preparation Kit helps ensure high recovery of viral DNA with less labor and error than manual processing. The Pharma KingFisher Apex instrument can process up to 24 samples in triplicate, as compared to 3 samples in triplicate using a manual method.

To help ensure performance that meets or exceeds regulatory compliance, the ViralSEQ kit has been internally validated on the Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System. Data analysis is streamlined using the QuantStudio Absolute Q dPCR Software, which includes accurate quantitation and security, audit, and e-signature (SAE) capabilities to help enable 21 CFR Part 11 compliance.

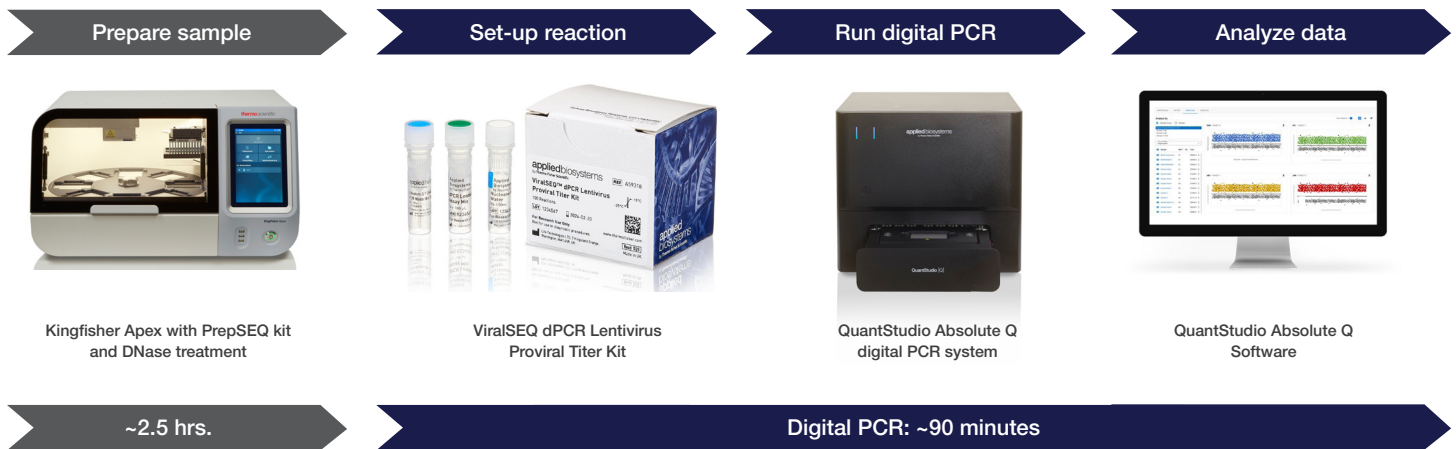


Figure 3. An integrated workflow solution to support process development and a good manufacturing practice (GMP) environment.



Powerfully simple digital PCR

The Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System offers an easy-to-use workflow, delivering results from DNA samples in <3 hours with minimal hands-on time. Moreover, there is no steep learning curve, as the workflow is nearly identical to that for real-time PCR.

- **Simple**—streamlined workflow integrates all dPCR steps into a single instrument
- **Fast**—the QuantStudio Absolute Q system requires only one hands-on step that takes <5 minutes to complete with minimal technical skill

Ordering information

Product	Quantity	Cat. No.
ViralSEQ dPCR Lentivirus Proviral DNA Titer Kit	100 reactions	A59318
Sample preparation and automation		
PrepSEQ Nucleic Acid Sample Preparation Kit	100 reactions	A50485
Pharma KingFisher Apex 96 Deep-Well Magnetic Particle Processor	1 instrument	A57715
System		
QuantStudio Absolute Q Digital PCR System	1 instrument	A52864
Absolute Q DNA Digital PCR Master Mix (5X)	200 reactions	A52490
Service		
QuantStudio Absolute Q IQ/OQ Service	1 service	A53878
QuantStudio Absolute Q CSV Service 1 service A55623	1 service	A55623
Pharma KingFisher Apex IQ/OQ Service	1 service	A31532
Related products		
ViralSEQ dPCR Lentivirus Physical DNA Titer Kit	100 reactions	A59317

 Learn more at thermofisher.com/viralseq

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