applied biosystems

Lentivirus quantitation

ViralSEQ Lentivirus Proviral DNA Titer Kit

Integrated sample preparation and RT-qPCR assay for quantitation of lentiviral proviral DNA in transduced cells

The Applied Biosystems™ ViralSEQ™ Lentivirus Proviral DNA Titer Kit offers:

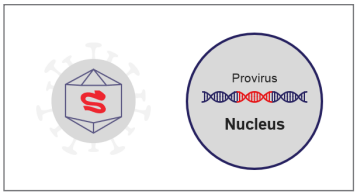
- Accurate quantitation of integrated genome copies by targeting a conserved region in the lentivirus genome
- Assay design compatibility with more than 200 lentiviral transfer plasmids
- Highly sensitive quantitation using proven Applied Biosystems™ TaqMan™ qPCR technology, with results in as few as 5 hours
- Manual and automated sample preparation, optimized for quantitative DNA recovery from transduced cells
- Easy-to-use, integrated sample-to-results system with sample preparation kit, master mix, TaqMan primer and probe mixes, and a DNA standard

With the ViralSEQ 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 Lentivirus Proviral DNA Titer Kit provides a wide dynamic range and high sensitivity for accurate detection of integrated provirus copies (Figures 1–3).

Table 1. ViralSEQ Lentivirus Proviral DNA Titer Kit performance specifications

Specification	
Linearity	R^2 ≥0.99
PCR efficiency	100% ± 10%
Precision	Back-calculated coefficient of variation (CV) ≤30%
Limit of detection (LOD)	10 copies per reaction
Limit of quantitation (LOQ)	25 copies per reaction
Assay range	25 to 10^7 genome copies per reaction

The broad dynamic range and high sensitivity provided by the ViralSEQ Lentivirus Proviral DNA Titer assay make it suitable for proviral quantitation in process development and lot release (Table 1).



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

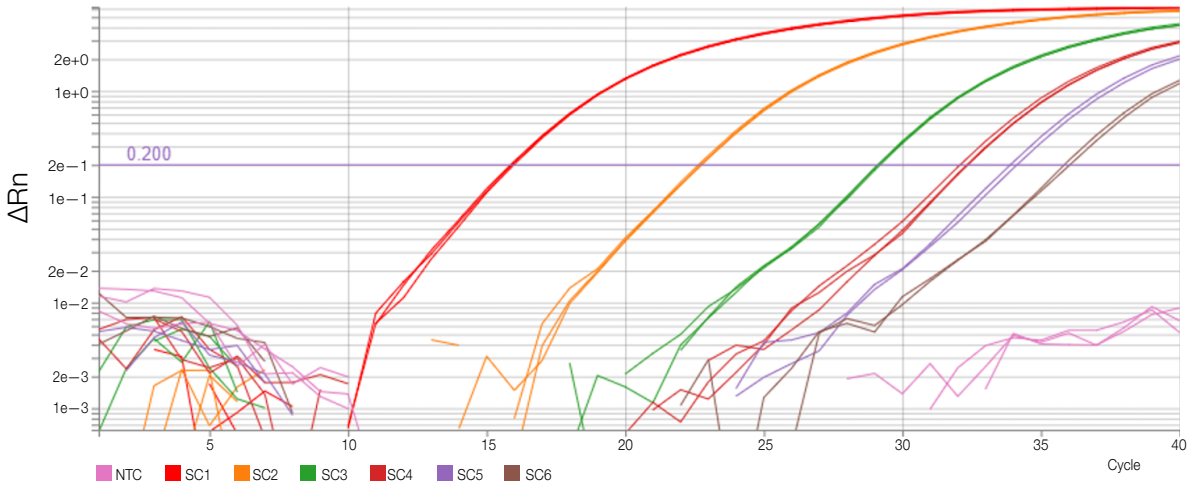
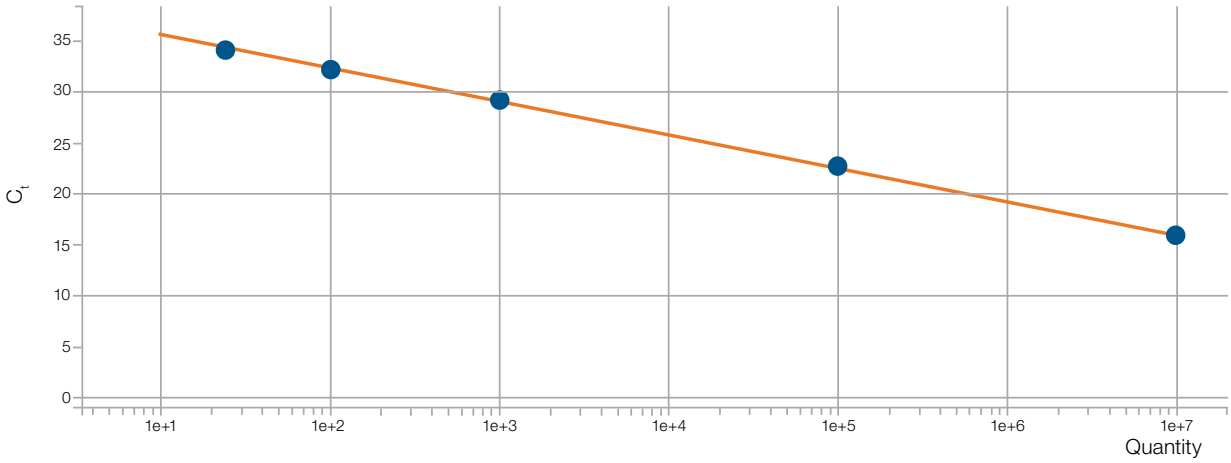


Figure 1. High sensitivity and broad dynamic range. The amplification plots were generated after preparing dilutions of standard provided in the kit ranging from 10 copies to 10^7 copies.



Target: Lentivirus Proviral DNA, Slope: -3.2403, R^2: 0.9994, Y-Intercept: 38.7395, Efficiency: 103.52, StdError: 0.0227

Figure 2. Standard curve for ViralSEQ Lentivirus Proviral DNA Titer Kit. The data generated an R^2 of 0.9994 with an efficiency of 103.52%.

	ViralSEQ kit	SYBR assay
HEK293		
CHO		
E. coli		
Sf9		
MDCK		
Baculovirus		
AmpR		
KanR		
AAV6		
pAV1		
Bovine		

No cross-reactivity Cross-reactive

Figure 3. The ViralSEQ kit is highly specific to lentivirus proviral DNA. 3 ng of potential cross-reactive DNA common to bioproduction environments was spiked into the reaction mix. The ViralSEQ Lentivirus Proviral DNA titer kit showed no cross-reactivity when compared to an alternative SYBR-based qPCR kit.

The ViralSEQ Lentivirus Proviral DNA Titer Kit is part of an integrated workflow for viral vector quantitation during biopharmaceutical manufacturing (Figure 4). Using the Applied Biosystems™ Pharma KingFisher™ Flex 96 Deep-Well Magnetic Particle Processor with the Applied Biosystems™ PrepSEQ™ Nucleic Acid Sample Preparation Kit helps ensure high recovery of genomic DNA with less labor and error than manual processing. The Pharma KingFisher Flex 96 instrument can process up to 24 samples in triplicate, compared to 3 samples in triplicate using the manual method. For qPCR, the ViralSEQ kit has been validated on the Applied Biosystems™ 7500 Fast Real-Time PCR System and the QuantStudio™ 5 Real-Time PCR System. Data analysis is streamlined using Applied Biosystems™ AccuSEQ™ Real-Time PCR Detection Software, which includes accurate quantitation and security, audit, and e-signature (SAE) capabilities to help enable 21 CFR Part 11 compliance.

Figure 4. Integrated workflow solution to support process development and good manufacturing practice (GMP) environments.

Ordering information

Product	Quantity	Cat. No.
ViralSEQ Lentivirus Proviral DNA Titer Kit	100 reactions	18765445
ViralSEQ Lentivirus Proviral DNA Titer Kit with PrepSEQ Nucleic Acid Sample Prep Kit	100 reactions	18715505
Sample preparation and automation		
PrepSEQ Nucleic Acid Sample Preparation Kit	100 reactions	18755475
Pharma KingFisher Flex 96 Deep-Well Magnetic Particle Processor	1 instrument	Speak with a representative
System		
QuantStudio 5 Real-Time PCR System, 96-well, 0.1 mL, desktop	1 instrument	Speak with a representative
Software		
AccuSEQ Real-Time PCR Software v3.0	1 license	18773634
Service		
QuantStudio 5 IQ/OQ Service	1 service	18735455
Related products		
ViralSEQ Lentivirus Physical Titer Kit	100 reactions	18765435
ViralSEQ Lentivirus Physical Titer Kit with PrepSEQ Nucleic Acid Sample Preparation Kit	100 reactions	18725405

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