

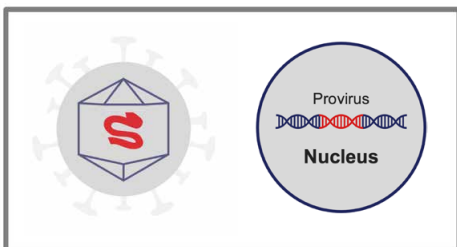
Lentivirus quantitation

ViralSEQ Lentivirus Physical Titer Kit

Integrated sample preparation and RT-qPCR assay for quantitation of lentiviral particles

- Accurate quantitation of viral particles by RT-qPCR targeting a conserved region in the lentivirus genome
- Assay design compatible with more than 200 lentivirus transfer plasmids
- Highly sensitive quantitation using proven Applied Biosystems™ TaqMan™ real-time PCR technology, with results in about 5 hours
- Manual and automated sample preparation, optimized for quantitative recovery from bioproduction samples
- Easy-to-use, integrated sample-to-results system with sample preparation kit, master mix, Applied Biosystems™ TaqMan™ primer/probe mixes, and lentiviral RNA standard

The Applied Biosystems™ ViralSEQ™ Lentivirus Physical Titer Kit is a one-step RT-qPCR assay for the reproducible quantitation of lentiviral particles in supernatants from cell-based bioproduction systems (Table 1). Lentiviral quantitation is a critical quality attribute (CQA) in viral vector manufacturing for cell therapy development. The assay targets a conserved region in the lentivirus genome and can be used with most lentiviral production systems. Compared to the p24 ELISA method, viral quantitation by RT-qPCR has a broader dynamic range and lower variance (Figure 1).



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



Table 1. ViralSEQ Lentivirus Physical Titer Kit performance specifications.

Specifications	
Linearity	$R^2 \geq 0.99$
PCR efficiency	100% \pm 10%
Precision	Back-calculated CV \leq 30%
Limit of detection (LOD)	10 copies per reaction
Limit of quantitation (LOQ)	50 copies per reaction
Assay range	50 to 10^9 copies per reaction

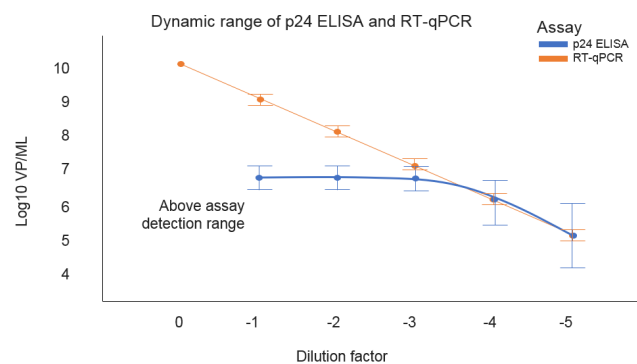


Figure 1. Viral quantitation by p24 ELISA and ViralSEQ RT-qPCR assays of a lentiviral vector sample at different concentrations. The p24 ELISA has a limited dynamic range and higher inter-run variation. Each error bar represents 1 standard deviation from the mean.

The broad dynamic range and high specificity provided by the ViralSEQ Lentivirus Physical Titer Kit make it suitable for viral quantitation in process development and lot release (Figures 2–4).

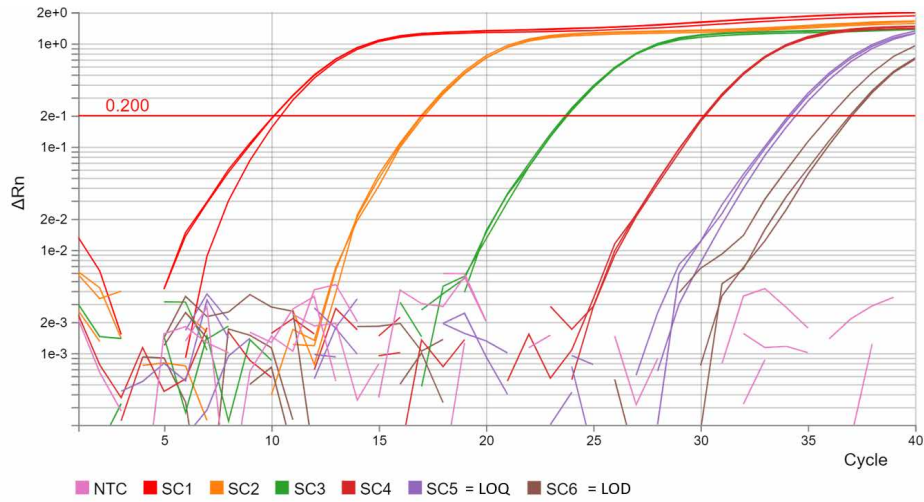


Figure 2. High sensitivity and broad dynamic range. The amplification plot was generated after preparing a dilution series of the standard provided in the kit, ranging from 10 copies to 10⁹ copies.

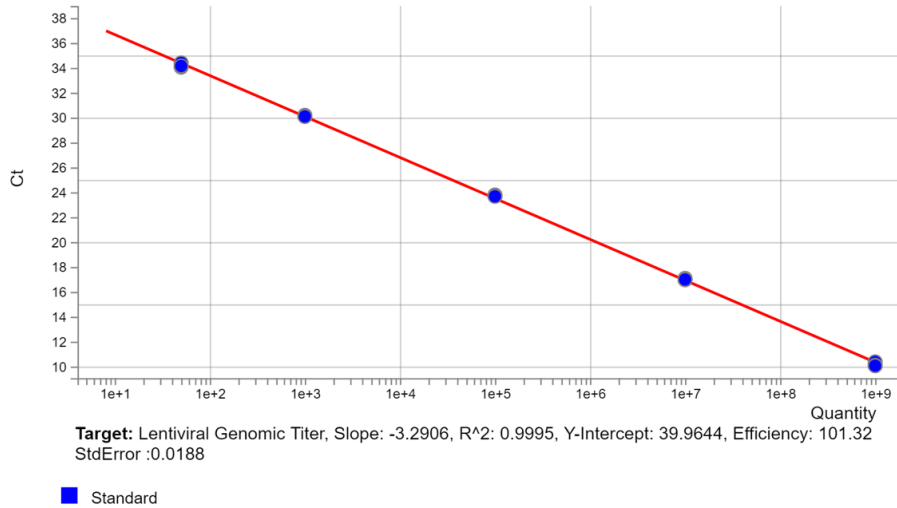


Figure 3. Standard curve for the ViralSEQ Lentivirus Physical Titer Kit. The data generated an R² of 0.9995 with an efficiency of 101.32%.

	Specificity	
	ViralSEQ kit	SYBR assay
HEK293	Grey	Red
CHO	Grey	Red
<i>E. coli</i>	Grey	Red
Sf9	Grey	Red
MDCK	Grey	Red
Baculovirus	Grey	Red
AmpR	Grey	Red
KanR	Grey	Red
AAV6	Grey	Red
pAV1	Grey	Red
Bovine	Grey	Red

No cross-reactivity
 Cross-reactivity

Figure 4. The ViralSEQ Lentivirus Physical Titer Kit is highly specific to lentivirus RNA. Reaction mixes were spiked with 3 ng of different types of potentially cross-reactive DNA common to bioproduction environments. The ViralSEQ Lentivirus Physical Titer Kit showed no cross-reactivity when compared to an alternative SYBR™ Green dye-based RT-qPCR kit.

The ViralSEQ Lentivirus Physical Titer Kit is part of an integrated workflow for viral vector characterization during biopharmaceutical manufacturing (Figure 5). Use of the Thermo Scientific™ Pharma KingFisher™ Flex 96 Deep-Well Magnetic Particle Processor with the Applied Biosystems™ PrepSEQ™ Nucleic Acid Sample Preparation Kit helps ensure high recovery of viral RNA 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 Applied Biosystems™ 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 capabilities to help enable 21 CFR Part 11 compliance.



Figure 5. An integrated workflow solution to support process development and a good manufacturing practice (GMP) environment.

Ordering information

Product	Quantity	Cat. No.
ViralSEQ Lentivirus Physical Titer Kit	100 reactions	A52597
ViralSEQ Lentivirus Physical Titer Kit with PrepSEQ Nucleic Acid Sample Preparation Kit	100 reactions	A52598
Sample preparation and automation		
PrepSEQ Nucleic Acid Sample Preparation Kit	100 reactions	A50485
DNase I, RNase-free (1 U/μL)	1,000 units	EN0521
Pharma KingFisher Flex 96 Deep-Well Magnetic Particle Processor	1 instrument	A31508
System		
QuantStudio 5 Real-Time PCR System, 96-well, 0.1 mL	1 instrument	A31672
Software		
AccuSEQ Real-Time PCR Detection Software 3.0	1 license	A48509
Service		
QuantStudio 5 IQ/OQ Service	1 service	A45613
Related products		
ViralSEQ Lentivirus Proviral DNA Titer Kit	100 reactions	A53561
ViralSEQ Lentivirus Proviral DNA Titer Kit with PrepSEQ Nucleic Acid Sample Preparation Kit	100 reactions	A53562