ThermoFisher SCIENTIFIC

The way forward in digital PCR

ViralSEQ dPCR AAV Titer Kit

Viral quantitation

Highly sensitive dPCR assay for quantitation of adenovirus associated particles

The Applied Biosystems™ ViralSEQ™ dPCR AAV Titer Kit is a digital PCR assay for the reproducible quantitation of AAV particles, independent of the serotype. AAV titer quantitation is a critical quality attribute (CQA) in viral vector manufacturing for gene therapy development. This probe-based, dPCR assay enables superior sensitivity and consistency, outperforming conventional DNA blotting or ELISA methods. (Figures 1–2).

- Accurate quantitation of viral particles by dPCR targeting ITR and SV40
- Assay design compatible with more than ~80% of AAV 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 supported by the ViralSEQ dPCR AAV Titer Kit make it suitable for viral quantitation in process development and lot release testing (Tables 1-3).

Table 1. ViralSEQ dPCR AAV Titer Kit performance specifications.

Specifications	
Linearity	$R^2 \geq 0.99$
PCR efficiency	100% ± 10%
Precision	<20%
Limit of detection (LOD)	9 copies per reaction
Limit of quantitation (LOQ)	27 copies / rxn (ITR), 13.5 copies / rxn (SV40)
Assay range	9 to 90,000 copies / rxn

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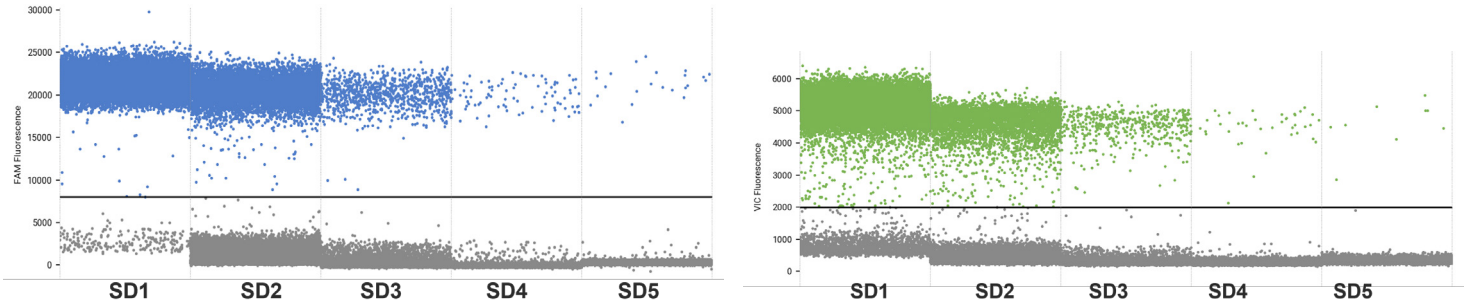

Figure 1. 1D dot plots displaying dPCR-based quantification of ITR & SV40 from serially diluted samples. Using the QuantStudio Absolute Q Digital PCR System, absolute quantification of AAV copies across serially diluted samples is possible by counting the total number of microchambers positive for the fluorescent label.

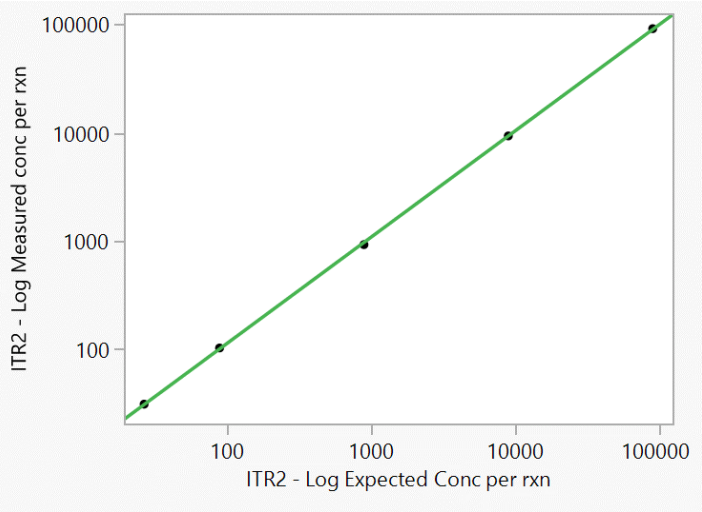

Figure 2. High sensitivity and broad dynamic range. Linear range was generated using serial dilutions ranging from 10,000 copies/µL (cp/µL) (SD1) to 3 copies/µL (SD5) of ITR2 DNA. Linearity: R2 >0.99; slope = 1.01.

Table 2. Summary of assay accuracy and precision of the resDNASEQ dPCR AAV Titer Kit. Accuracy measured against a known target.

Target	Dilution Step	Expected Conc. (cp/µL)	Ave. Conc. (cp/µL)	Relative Accuracy (%)	SD	CV%
ITR	SC1(ULOQ)	10,000.00	10527.1	105.27	90.95	0.86%
	SC3	100.00	110.1	110.11	4.58	4.16%
	SC4	10.00	11.9	118.68	0.61	5.15%
	SC5(LOQ)	3.00	3.5	116.07	0.14	3.95%
SV40	SC1(ULOQ)	5000	5330.4	106.61	34.81	0.65%
	SC3	50	55.6	111.27	1.76	3.17%
	SC4	5	6.0	119.04	0.41	6.86%
	SC5(LOQ)	1.5	1.7	115.33	0.17	10.02%

Table 3. Summary of assay LOD for both targets of the resDNASEQ dPCR AAV Titer Kit.

Target	Expected Conc. of LOD (cp/µL)	Ave. Conc. of LOD (cp/µL)	Ave. Conc. of NTC (cp/µL)	SD of NTC (cp/µL)	Conc. of NTC + 3 SD (cp/µL)
ITR	1.00	0.99	0.05	0.09	0.33
SV40	0.50	0.52	0.00	0.02	0.06

Digital PCR workflow

The ViralSEQ dPCR AAV 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.

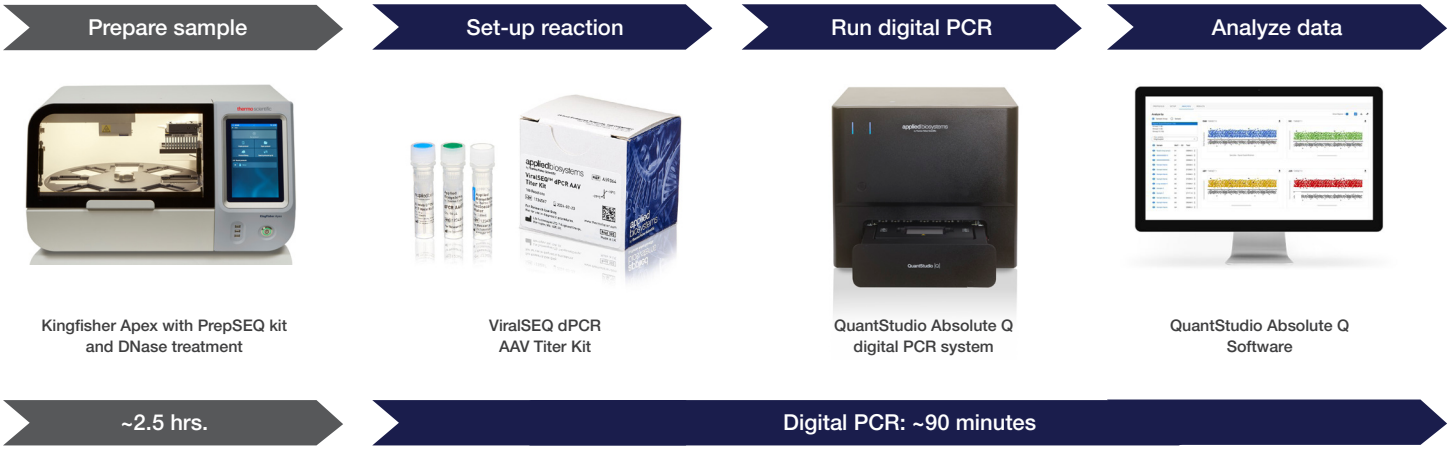

Figure 3. An integrated workflow solution to support process development and a good manufacturing practice (GMP) environment.

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 AAV Titer Kit	100 reactions	A59364
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		
ResDNASEQ dPCR E1A DNA Sizing Kit	100 reactions	A55852

Learn more at **thermofisher.com/viralseq**