Thermo Fisher
S C I E N T I F I C

The way forward in digital PCR

resDNASEQ dPCR Sf9/Baculovirus DNA Kit

Residual DNA quantitation

Highly sensitive dPCR assay for quantitation of residual Sf9/Baculovirus host-cell DNA

The Applied Biosystems™ resDNASEQ™ dPCR Sf9/Baculovirus DNA Kit is a powerful digital PCR (dPCR) solution for quantitating residual DNA from Sf9/Baculovirus expression systems, which are commonly utilized for the production of gene therapies, cell-based vaccines, and similar therapeutics. By harnessing the capabilities of high-performance Applied Biosystems™ TaqMan™ chemistry, the resDNASEQ Sf9/Baculovirus assay offers a rapid and reliable approach for accurately quantifying residual Sf9/Baculovirus DNA (Tables 1-2). This assay offers exceptional sensitivity and specificity, helping ensure that the quantitation data obtained

from a range of sample types, including in-process samples with varying sample matrices and final product formulations, is reliable and consistent.

- Accurate, absolute quantitation of residual Sf9/Baculovirus DNA

- 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 of samples from complex matrices

Table 1. Sensitive and specific quantitation of Sf9/Baculovirus using the ResDNASEQ dPCR Sf9/Bacoluvirus DNA Kit.

Specifications	
Linearity	$R^2 \geq 0.99$
Precision	<20%
Limit of detection (LOD)	1 copy/µL; 1.5fg/rxn for BAC and 24 fg for Sf9
Limit of quantitation (LOQ)	3 copies/µL; 4.5fg/rxn for BAC and 71 fg/rxn for Sf9
Slope	0.95-1.05

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The dPCR fluorescence plots (Figures 1-2) illustrate the sensitivity of the assay for both targets enabling accurate simultaneous quantitation of both impurities.
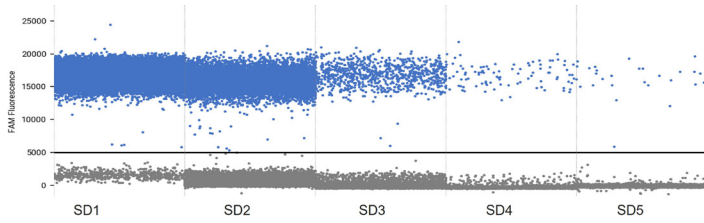

Figure 1. 1D dot plots displaying dPCR-based quantification of Baculovirus from serially diluted samples. The Baculovirus fluorescent plot was generated using serial dilutions ranging from 10,000 copies/µL (cp/µL) (SD1) to 3 copies/µL (SD5) of *Baculovirus DNA*.
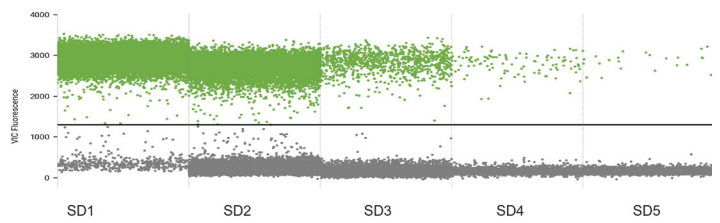
Figure 2. 1D dot plots displaying dPCR-based quantification of Sf9 DNA from serially diluted samples. The Sf9 fluorescent plot was generated using serial dilutions ranging from 10,000 copies/µL (cp/µL) (SD1) to 3 copies/µL (SD5) of *Sf9 DNA*.

The linear curves provided by TaqMan dPCR Assay technology allows testing of a wide range of samples containing Sf9/Baculovirus DNA (Figure 3).
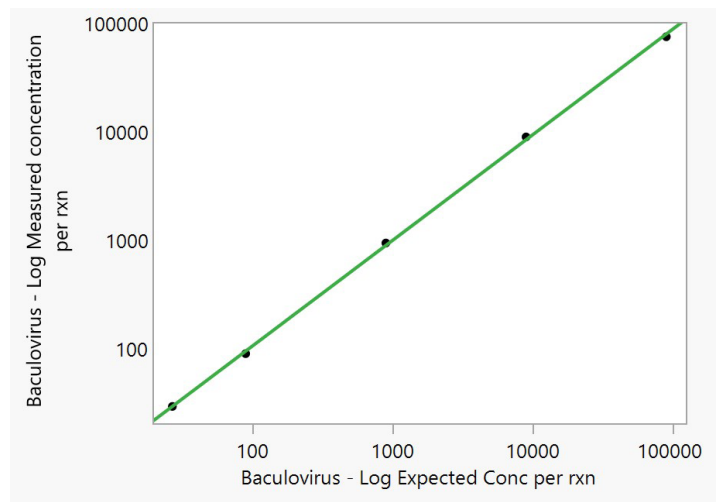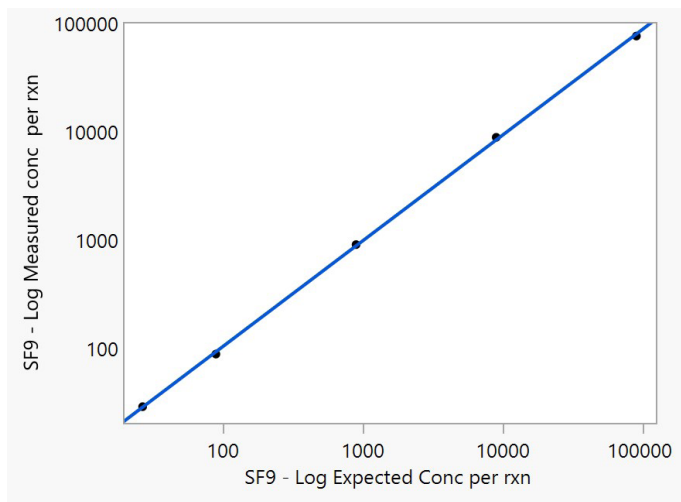
Figure 3. High sensitivity and broad dynamic range. Linear curves were generated using serial dilutions ranging from 10,000 copies/µL (cp/µL) (SD1) to 3 copies/µL (SD5) of *Sf9 and Baculovirus* DNA. Linearity: R2 >0.99; slope = 1.01.

Table 2. resDNASEQ dPCR Sf9/Baculovirus DNA Kit performance summary demonstrates assay accuracy and precision among triplicate reactions.

BAC	Expected Conc. (cp/µL)	Expected Conc. (fg/µL)	Expected Conc. (fg/rxn)	Ave. Conc. (cp/µL)	Ave. Conc. (fg/µL)	Relative Accuracy (%)	SD	CV%
SC1(ULOQ)	10,000.00	**1663.62**	14972.6	9138.5	**1520.30**	91.38	157.2	1.72%
SC3	100	**16.64**	149.7	103.5	**17.22**	103.48	3.7	3.54%
SC4	10	**1.66**	15.0	10.2	**1.70**	101.53	0.3	2.65%
SC5(LOQ)	3	**0.50**	4.5	2.8	**0.47**	92.83	0.1	4.62%

Sf9	Expected Conc. (cp/µL)	Expected Conc. (fg/µL)	Expected Conc. (fg/rxn)	Ave. Conc. (cp/µL)	Ave. Conc. (fg/µL)	Relative Accuracy (%)	SD	CV%
SC1(ULOQ)	10,000.00	26109.66	**234986.9**	9993.7	**26093.21**	99.94	273.9	2.74%
SC3	100	261.10	**2349.9**	113.3	**295.82**	113.32	6.4	5.68%
SC4	10	26.11	**235.0**	11.2	**29.24**	112.15	0.2	1.70%
SC5(LOQ)	3	7.83	**70.5**	3.6	**9.40**	120	0.2	4.74%

Table 3. Summary of assay LOD for both targets of the resDNASEQ dPCR Sf9 and Baculovirus DNA Kit.

Test Item	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)
Bac	1.00	1.06	0.02	0.04	0.14
Sf9	1.00	1.19	0.00	0.00	0.00

Digital PCR workflow

The resDNASEQ dPCR Sf9/Baculovirus DNA Kit is part of an integrated digital PCR workflow for impurity testing during biopharmaceutical manufacturing (Figure 4). 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 residual 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 resDNASEQ 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.

Prepare sample	Set-up reaction	Run digital PCR	Analyze data
Kingfisher Apex with PrepSEQ kit and DNase treatment	resDNASEQ dPCR Sf9/ Baculovirus DNA Kit	QuantStudio Absolute Q digital PCR system	QuantStudio Absolute Q Software

~2.5 hrs. — Digital PCR: ~90 minutes

Figure 4. 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.
resDNASEQ dPCR Sf9/Baculovirus DNA Kit	100 reactions	A59319
Sample preparation and automation		
PrepSEQ Residual DNA Sample Preparation Kit	100 reactions	4413686
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

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