ViralSEQ Quantitative Sf-Rhabdovirus Kit

Negative sense–specific RT-qPCR assay for accurate quantitation of Sf-rhabdovirus genomic RNA

- Accurate quantitation of Sf-rhabdovirus genomic RNA utilizing a strand-specific design
- No cross-reactivity with Sf-rhabdovirus mRNA or non-target species
- Highly sensitive quantitation using proven Applied Biosystems[™] TaqMan[®] real-time PCR technology
- Manual and automated sample preparation optimized for reproducible recovery of RNA from common bioproduction sample matrices
- Easy to use, with results in under six hours
- Integrated sample-to-results system with sample preparation kit, master mix, Applied Biosystems[™] TaqMan[®] primer/probe mix, and RNA standard

The Applied Biosystems[™] ViralSEQ[™] Quantitative Sf-Rhabdovirus Kit is a two-step RT-qPCR assay for the quantitation of Sf-rhabdovirus RNA from the Sf9 and Sf21 baculovirus platforms used in the development of gene therapies, cell-based vaccines, and other biotherapeutics. This assay leverages a tagged reverse transcription (RT) primer design containing a nonviral sequence to enable sensitive and specific quantitation of negative-sense genomic Sf-rhabdovirus RNA (Table 1, Figure 1). This design allows more accurate quantitation of true viral counts and prevents overestimation that is usually due to mRNA in non-strand–specific assay designs.



Table 1. Sensitive and specific quantitation of Sf-rhabdovirus RNA using the ViralSEQ Quantitative Sf-Rhabdovirus Kit.

Specification		
Linearity	R ² > 0.99	
PCR efficiency	100% ± 10%	
Precision	≤10% CV	
Limit of detection (LOD)	10 copies	
Limit of quantitation (LOQ)	30 copies	
Assay range	10 to 1 x 10 ⁷ copies	



The complete workflow for quantitation of Sf-rhabdovirus genomic RNA can be performed in under six hours (Figure 1). A wide range of samples can be tested due to the broad linear range provided by TaqMan[®] real-time PCR technology (Figures 2 and 3).



Figure 1. Workflow overview for the ViralSEQ Quantitative Sf-Rhabdovirus Kit.



S1 S2 S3 S4 S5 S6 NTC

S1	1E+07 copies/reaction
S2	1E+05 copies/reaction
S3	1000 copies/reaction
S4	100 copies/reaction
S5	30 copies/reaction (LoQ)
S6	10 copies/reaction (LoD)
NTC	No template control/NTC

Figure 2. High sensitivity and broad dynamic range. The amplification plots were generated after preparing serial dilutions ranging from 10 copies to 10^7 copies using the RNA standard provided in the kit.



Figure 3. Standard curve for a 10-fold dilution series. The data generated an R^2 of 0.9991 with an efficiency of 99.71%.

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ViralSEQ Quantitative Sf-Rhabdovirus Kit workflow



Figure 4. Integrated workflow solution to support process development and GMP environments. The ViralSEQ Quantitative Sf-Rhabdovirus Kit is part of an integrated workflow for impurity and contaminant testing during biopharmaceutical manufacturing. Using a Thermo Scientific[™] Pharma KingFisher[™] Flex 96 Deep-Well Magnetic Particle Processor and an Applied Biosystems[™] PrepSEQ[™] Nucleic Acid Sample Preparation Kit provides high RNA recoveries with less labor and error. The Pharma KingFisher Flex system can process up to 24 samples in triplicate compared to just three samples in triplicate using the manual method. The reverse transcription step is performed on a Veriti 96-Well Fast Thermal Cycler (0.1 mL block configuration). The ViralSEQ kit has been validated for qPCR on an Applied Biosystems[™] 7500 Fast Real-Time PCR System and a QuantStudio 5 Real-Time PCR System (0.1 mL block configuration). Data analysis is streamlined with Applied Biosystems[™] AccuSEQ[™] Real-Time PCR Detection Software. The software provides accurate quantitation, security, and audit and e-signature capabilities to support 21 CFR Part 11 compliance.

Ordering information

Product	Quantity	Cat. No.
ViralSEQ Quantitative Sf-Rhabdovirus Kit	100 reactions	A50484
ViralSEQ Quantitative Sf-Rhabdovirus Kit with PrepSEQ Nucleic Acid Sample Preparation Kit	100 reactions	A50496
Sample preparation and automation		
PrepSEQ Nucleic Acid Sample Preparation Kit	100 reactions	A50485
Pharma KingFisher Flex 96 Deep-Well Magnetic Particle Processor	1 instrument	A31508
System		
QuantStudio 5 Real-Time PCR System (0.1 mL block configuration)	1 instrument	A31672
7500 Fast Real-Time PCR System with Dell Tower	1 instrument	4365463
Veriti 96-Well Fast Thermal Cycler (0.1 mL block configuration)	1 instrument	4375305
Software		
AccuSEQ Real-Time PCR Detection Software	1 license	A48509
Service		
QuantStudio 5 IQ/OQ Service	1 service	A45613
Pharma KingFisher Flex IQ/OQ	1 service	A31532

Find out more at thermofisher.com/viralseq



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