

Sf9 & AcNPV DNA Residue Detect

Product description

The Sf9 and Baculovirus (AcNPV) DNA Residual Detection Kit is designed for quantitative analysis of residual host cell DNA from Sf9 insect cells and residual baculovirus (AcNPV) DNA in biopharmaceutical products developed or manufactured using the baculovirus-insect cell (Sf9) expression system, including gene therapies, recombinant proteins, and vaccines.

This kit is based on probe-based real-time PCR (qPCR) technology and employs a multiplex qPCR method to separately detect residual Sf9 and AcNPV DNA. The quantification limit reaches 1 fg/ μ L for Sf9 DNA and 7 copies/ μ L for AcNPV DNA. This kit can be used in combination with our magnetic bead-based residual DNA sample pretreatment kits (Cat#18461ES).

Specifications

Cat NO.	41330ES50 / 41330ES60
Size	50 T / 100 T

Components

Components No.	Name	41330ES50	41330ES60
41330-A	Sf9&AcNPV qPCR Mix	0.75 mL	1.5 mL
41330-B	Sf9&AcNPV Primer&Probe Mix	200 μ L	400 μ L
41330-C	DNA Dilution Buffer	1.8 mL $\times$ 2 tubes	1.8 mL $\times$ 4 tubes
41330-D	Sf9&AcNPV DNA Control Mix (30 ng/ μ L & 2.12×10^8 copies/ μ L)	25 μ L	50 μ L
41330-E	IC*	50 μ L	100 μ L

* IC: Internal Control

Storage

1. All components are shipped on dry ice and should be stored at -25°C to -15°C . The shelf life is 2 years. Components 41330-A and 41330-B must be protected from light.
2. Upon receipt, please verify that all 5 components are present and immediately store them at the specified temperature.

Compatible Instruments

Including but not limited to:

Thermo Scientific: ABI 7500, ABI Quant Studio 5

Bio-Rad: CFX96 Optic Module

Bioer: LineGene 9600

Shanghai Holmes Biomedical Technologies: SLAN-96S

Instructions

1. Dilution of Sf9 & AcNPV DNA Control Mix and Preparation of Standard Curve

The concentration of the quantitative reference standard is labeled on the tube. Sf9 DNA Control concentration: 30 ng/μL; AcNPV DNA Control concentration: 2.12×10^8 copies/μL.

Procedure:

- 1) Thaw the Sf9&AcNPV DNA Control Mix (quantitative reference standard) and DNA Dilution Buffer at room temperature. After complete thawing, gently vortex to mix and briefly centrifuge for 10 seconds.
- 2) Label seven clean 1.5 mL microcentrifuge tubes as Std0, Std1, Std2, Std3, Std4, Std5, and Std6.
- 3) Add 90 μL of DNA Dilution Buffer and 10 μL of Sf9&AcNPV DNA Control Mix into the tube labeled Std0, resulting in a 10-fold dilution (Sf9: 3 ng/μL, AcNPV: 2.12×10^7 copies/μL). Vortex thoroughly and briefly centrifuge for 10 seconds. This Std0 solution can be aliquoted and stored at -25°C to -15°C for short-term use (up to 1 year)**. Avoid repeated freeze-thaw cycles during use.
- 4) Add 90 μL of DNA Dilution Buffer to each of the tubes labeled Std1–Std6, then perform serial dilutions as described below:

Table 1. Serial Dilution of Standards

Dilution Tube	Dilution Ratio	Final Concentration	
		Sf9	AcNPV
Std1	10 μL Std0 + 90 μL DNA Dilution Buffer	300 pg/μL	2.12×10^6 copies /μL
Std2	10 μL Std1 + 90 μL DNA Dilution Buffer	30 pg/μL	2.12×10^5 copies /μL
Std3	10 μL Std2 + 90 μL DNA Dilution Buffer	3 pg/μL	2.12×10^4 copies /μL
Std4	10 μL Std3 + 90 μL DNA Dilution Buffer	300 fg/μL	2.12×10^3 copies /μL
Std5	10 μL Std4 + 90 μL DNA Dilution Buffer	30 fg/μL	2.12×10^2 copies /μL
Std6	10 μL Std5 + 90 μL DNA Dilution Buffer	3 fg/μL	2.12×10^1 copies /μL

[Note]:* Each concentration should be tested in triplicate. The linear range may be adjusted if necessary.

** To minimize freeze-thaw cycles and contamination, it is recommended to aliquot the DNA reference standard upon first use and store at -25°C to -15°C.

*** Thawed but unused DNA Dilution Buffer can be stored at 2–8°C for up to 7 days. For long-term storage, keep at -25°C to -15°C.

**** To ensure complete mixing, vortex gently for approximately 1 minute at each dilution step.

2. Preparation of Extraction Recovery Control (ERC)

Prepare ERC with desired concentrations of Sf9 and AcNPV DNA standards (example: adding 30 pg Sf9 DNA corresponding to 2.12×10^5 copies of AcNPV DNA):

- 1) Add 100 μL of the test sample to a clean 1.5 mL microcentrifuge tube, then add 10 μL of Std3. Mix well and label as ERC.
- 2) Process the ERC sample and the corresponding test samples together through the sample pretreatment procedure to prepare purified ERC solution.

3. Preparation of Negative Control Solution (NCS)

Set up NCS as needed:

1) Add 100 μL of sample matrix solution (or DNA Dilution Buffer) into a clean 1.5 mL microcentrifuge tube and label as NCS.

2) Process the NCS sample together with the test samples through the sample pretreatment procedure to prepare purified NCS solution.

4. Preparation of No Template Control (NTC)

Set up NTC as needed:

1) NTC does not require sample pretreatment; it is prepared at the qPCR detection stage.

2) The NTC reaction mixture per tube/well is: 20 μL Mix (15 μL Sf9&AcNPV qPCR Mix + 4 μL Sf9&AcNPV Primer&Probe Mix + 1 μL IC) + 10 μL DNA Dilution Buffer. It is recommended to prepare triplicate wells.

5. Reaction System

Table 2. Reaction Setup

Component	Volume (μL)
Sf9&AcNPV qPCR Mix*	15
Sf9&AcNPV Primer&Probe Mix	4
IC	1
DNA Template**	10
Total Volume*	30

[Note]:* Calculate total Mix volume based on number of reaction wells:

Total Mix = (Number of reaction wells + 2) $\times$ (15 + 4 + 1) μL (including 2 wells for loss). Typically, each sample is run in triplicate.

** Number of reaction wells = (6 standard curve concentrations + 1 NTC + 1 NCS + number of test samples TS + number of ERC samples) $\times$ 3.

Definitions:

NTC (No Template Control): DNA Dilution Buffer

NCS (Negative Control Solution): Sample matrix or DNA Dilution Buffer after sample pretreatment, resulting in purified NCS

TS (Test Sample): Test sample

ERC (Extraction Recovery Control): Test sample spiked with 10 μL of 3 pg/ μL standard DNA, followed by sample pretreatment to yield purified ERC

*** After adding all components and sealing the tubes, briefly centrifuge at low speed for 10 seconds to collect liquid from tube walls. Then vortex mix for at least 5 seconds to ensure complete homogenization, followed by another brief centrifugation to collect liquid. Remove any bubbles before loading.

Table 3. Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC		TS1	TS1	TS1		Std1	Std1	Std1			
B	NTC		TS2	TS2	TS2		Std2	Std2	Std2			
C	NTC		TS3	TS3	TS3		Std3	Std3	Std3			
D							Std4	Std4	Std4			

	1	2	3	4	5	6	7	8	9	10	11	12
E	NCS		ERC1	ERC1	ERC1		Std5	Std5	Std5			
F	NCS		ERC2	ERC2	ERC2		Std6	Std6	Std6			
G	NCS		ERC3	ERC3	ERC3							
H												

This example illustrates a qPCR assay setup for the Sf9 and Baculovirus (AcNPV) DNA Residual Detection Kit, including: 6-point standard curve, 1 NTC, 1 NCS, 3 test samples (TS), and 3 spiked recovery samples (ERC). Each sample is tested in triplicate.

6. Amplification Program Parameters (Two-Step Cycling) – Example using ABI 7500 qPCR Instrument, Software Version 2.0

1) Create a new blank program and select the absolute quantification template.

2) Create two detection probes:

Target 1: Name "Sf9", Reporter dye: "FAM", Quencher: "None"

Target 2: Name "AcNPV", Reporter dye: "VIC", Quencher: "None"

Create a third probe: Name "IC", Reporter dye: "CY5", Quencher: "None"

Set reference dye to "ROX" (reference dye selection may vary depending on instrument model).

3) In the "Assign target(s) to the selected wells" panel:

For standard curve wells: Set "Task" to "Standard". Assign "Quantity" values:

FAM channel: 300000, 30000, 3000, 300, 30, 3 (representing Sf9 DNA concentration per well in fg/μL)

VIC channel: 2.12×10^6 , 2.12×10^5 , 2.12×10^4 , 2.12×10^3 , 2.12×10^2 , 2.12×10^1 (representing AcNPV DNA concentration per well in copies/μL)

Label corresponding "Sample Name" as Std1, Std2, Std3, Std4, Std5, Std6.

For NTC wells: Set "Task" to "NTC".

For NCS, TS, and ERC wells: Set "Task" to "Unknown", and label "Sample Name" as "NCS", "TS", "ERC" respectively. Select "ROX" as reference dye.

Click "Start Run" to begin the run.

4) Amplification program settings: Three-step cycling, reaction volume 30 μL.

Table 4. Amplification Program

Cycle Step	Temperature (°C)	Time	Cycles
Pre-denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	45
Annealing/Extension (fluorescence collection)	60°C	30 sec	

7. qPCR Data Analysis

1) In the "Analysis" → "Amplification Plot" panel, the system automatically sets the "Threshold". If the threshold is too close to the baseline, causing large Ct variation among replicates, manually adjust the threshold to an appropriate position, then click "Analyze". Use the "Multicomponent Plot" to preliminarily assess the shape of amplification curves.

- 2) In the "Analysis" → "Standard Curve" panel, review R^2 , amplification efficiency (Eff%), slope, and intercept. Acceptable standard curve criteria: $R^2 > 0.99$, amplification efficiency between 90% and 110%, slope between -3.6 and -3.1.
- 3) In the "Analysis" → "View Well Table" panel, read the "Quantity" values (in fg/ μ L and copies/ μ L) for NTC, NCS, TS, and ERC. These values can be converted to other required units in the final report.
- 4) Data analysis parameters should be set according to the specific instrument and software version, though automatic analysis is typically available.
- 5) Calculate the spike recovery rate using the results from TS and ERC. Acceptable recovery range: 50%–150%.
$$\text{Recovery (\%)} = \frac{(\text{ERC measured value (e.g., pg/\mu L)} - \text{TS measured value (e.g., pg/\mu L)}) \times \text{elution volume (e.g., \mu L)}}{\text{theoretical amount of DNA added (e.g., pg)}} \times 100\%$$
- 6) The Ct value of NCS should be greater than the average Ct value of the lowest standard concentration.
- 7) The NTC result should be "Undetermined" or have a Ct value ≥ 35 .
- 8) The Ct-IC value of test samples should be consistent with that of NTC (within ± 1). A significantly higher Ct-IC in test samples compared to NTC indicates potential sample inhibition. If ERC samples are also tested, prioritize the spike recovery result; IC data serves as a secondary reference.

Notes

1. This product is intended for research use only.
2. For safety and health, wear a lab coat and disposable gloves during operation.
3. Read the manual carefully before use. All procedures, including sample handling, reaction setup, and pipetting, should follow standard laboratory practices.
4. Vortex each component thoroughly and briefly centrifuge before use.