

Sf9 & AcNPV DNA Residue Detection Kit

Performance Validation Summary (Catalog No.: 41330ES60)

Background

The Sf9 and Baculovirus (AcNPV) DNA Residue Detection Kit is independently developed by YEASEN Biotechnology (Shanghai) Co., Ltd. It is designed for quantitative analysis of residual DNA from Sf9 host cells and baculovirus (AcNPV) in various biopharmaceutical products—such as gene therapies, recombinant proteins, and vaccines—produced using the baculovirus-insect cell (Sf9) expression system. Based on probe-based fluorescent quantitative PCR, this kit employs a multiplex qPCR method to detect Sf9 and baculovirus (AcNPV) residual DNA separately. The limit of quantitation (LOQ) for Sf9 is 1 fg/μL, and for baculovirus (AcNPV), it is 7 copies/μL.

The data in this summary report are provided by YEASEN Biotechnology (Shanghai) Co., Ltd. Validation parameters include linearity, range, accuracy, precision, specificity, limit of quantitation, robustness, stability, and limit of blank. This summary is for reference only. Users should perform their own method validation for Sf9 and baculovirus (AcNPV) DNA residue testing according to their specific experimental conditions, sample types, and regulatory or pharmacopoeia requirements.

Materials and Methods

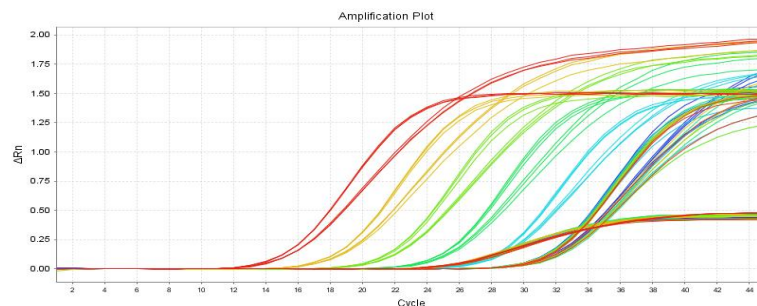
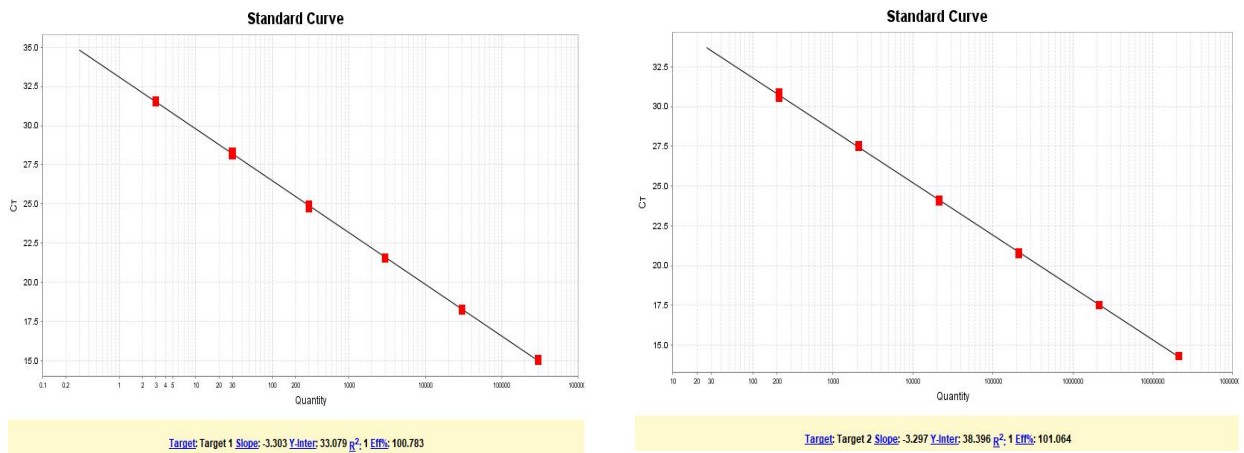
1. Sf9 and Baculovirus (AcNPV) DNA Residue Detection Kit: Cat#41330ES60 (YEASEN)
2. Magnetic Bead-Based Residual DNA Sample Pretreatment Kit: Cat#18461ES (YEASEN)
3. Raw Data: Electronically recorded in the sub-file "Product Experimental Report" under the master file "Sf9 and Baculovirus (AcNPV) DNA Residue Detection Kit"
4. Method: All materials and procedures were developed and established by YEASEN Biotechnology (Shanghai) Co., Ltd., with R&D and manufacturing processes strictly following ISO13485 requirements. Performance meets the relevant requirements of ICH Q2(R2) and the Chinese Pharmacopoeia 2020 Edition, Volume III, "Guidance on Validation of Analytical Methods 9101."

Validation Content and Results

1. Detection Range

Sf9 DNA linear range: 3 fg/μL to 300 pg/μL, $R^2 = 1$, amplification efficiency = 100.78%, CV of detection values at all concentrations < 15%

Baculovirus DNA linear range: 2.12×10^1 to 2.12×10^6 copies/μL, $R^2 = 1$, amplification efficiency = 101.06%, CV of detection values at all concentrations < 15%



2. Accuracy

2.1 Recovery Rate

2.1.1 Blank Sample Spiking Recovery Experiment

High, medium, and low concentrations were prepared: 300 pg/μL, 3 pg/μL, 30 fg/μL Sf9 DNA standards and 2.12×10^6 copies/μL, 2.12×10^4 copies/μL, 2.12×10^2 copies/μL AcNPV DNA standards. Samples were extracted using the magnetic bead-based pretreatment kit and tested (2 extraction replicates per sample, 3 test replicates per extraction). Recovery rate and CV were analyzed. Recovery rates for DNA samples at different concentrations were within 70%–130%, and CV < 20%.

Table 1. Blank Sample Spiking Recovery Rate

Sample Type	Gene	Theoretical Concentration	Mean of Extraction 1	Mean of Extraction 2	Average Measured Concentration	CV	Recovery Rate
Blank Sample	Sf9	/	/	/	/	/	/
	AcNPV	/	/	/	/	/	/
Recovered Sample 1	Sf9	300 pg/μL	278.93	282.75	280.84	0.96%	93.61%
	AcNPV	2.12×10^6 copies/μL	1.96×10^6	1.99×10^6	1.975×10^6	1.07%	93.16%
Recovered Sample 2	Sf9	3 pg/μL	2.88	2.87	2.875	0.25%	95.83%
	AcNPV	2.12×10^4 copies/μL	2.1×10^4	2.18×10^4	2.14×10^4	2.64%	100.94%
Recovered	Sf9	30 fg/μL	30.96	30.64	30.8	0.73%	102.67%

Sample Type	Gene	Theoretical Concentration	Mean of Extraction 1	Mean of Extraction 2	Average Measured Concentration	CV	Recovery Rate
Sample 3	AcNPV	2.12×10^2 copies/ μ L	1.98×10^2	1.87×10^2	1.925×10^2	4.04%	90.80%

2.1.2 Simulated Sample Spiking Recovery Experiment

Five different background solutions (high protein, high nucleic acid, high salt, high pH, low pH) were used to spike the same concentration sample (300 fg/ μ L Sf9 DNA, 2.12×10^4 copies/ μ L AcNPV DNA). Each background solution had 2 extraction replicates, with 3 test replicates per extraction. Recovery rate and CV were analyzed. Recovery rates for DNA samples in different background solutions were within 70%–130%, and CV < 20%.

Table 2. Simulated Sample Spiking Recovery Rate

Sample Type	Gene	Theoretical Concentration	Mean of Extraction 1	Mean of Extraction 2	Average Measured Concentration	CV	Recovery Rate
Base Sample	Sf9	/	/	/	/	/	/
	AcNPV	/	/	/	/	/	/
High Protein	Sf9	Sf9 300 fg/ μ L & AcNPV 2.12×10^4 copies/ μ L	268.48	279.53	274.01	2.85%	91.34%
	AcNPV		2.02×10^4	1.96×10^4	1.99×10^4	2.13%	93.87%
High Nucleic Acid	Sf9		257.6	261.87	259.74	1.16%	86.58%
	AcNPV		1.92×10^4	2.01×10^4	1.97×10^4	3.24%	92.69%
High Salt	Sf9		272.92	283.49	278.21	2.69%	92.74%
	AcNPV		1.95×10^4	1.99×10^4	1.97×10^4	1.44%	92.92%
High pH	Sf9		307.41	293.62	300.52	3.24%	100.17%
	AcNPV		2.21×10^4	2.08×10^4	2.15×10^4	4.29%	101.18%
Low pH	Sf9		285.54	290.23	287.89	1.15%	95.96%
	AcNPV		2.07×10^4	1.91×10^4	1.99×10^4	5.69%	93.87%

3. Precision

3.1 Repeatability

The standard samples of Sf9 DNA at 3 fg/ μ L and AcNPV baculovirus DNA at 2.12×10^1 copies/ μ L (i.e., 21.2 copies/ μ L) were tested 10 times. CV was < 15%, meeting repeatability requirements.

Table 3. Repeatability Test Results

Repetition	1	2	3	4	5	6	7	8	9	10	Mean	CV
Sf9 Detection Value (fg/ μ L)	2.41	2.97	2.92	2.54	2.47	2.51	2.63	2.50	2.85	2.42	2.62	8.09%
AcNPV Detection Value (copies/ μ L)	20.42	18.82	21.66	19.15	20.36	21.08	22.84	19.94	23.64	22.82	21.07	7.77%

3.2 Intermediate Precision

Three operators independently tested high, medium, and low concentrations: Sf9 DNA at 30 pg/μL, 300 fg/μL, 3 fg/μL, and AcNPV baculovirus DNA at 2.12×10^5 copies/μL, 2.12×10^3 copies/μL, 2.12×10^1 copies/μL. Each concentration had 3 replicates. The CV of the 9 resulting data points was < 15%.

Table 4. Intermediate Precision Test Results

Operator	Test Theoretical Concentration	Sf9 (pg/μL)			AcNPV (copies/μL)		
		30 pg/μL	300 fg/μL	3 fg/μL	2.12×10^5	2.12×10^3	2.12×10^1
Operator 1	Test 1	34.41	300.98	3.25	2.26×10^5	2.19×10^3	1.99×10^1
	Test 2	35.80	300.98	3.22	2.29×10^5	2.16×10^3	2.06×10^1
	Test 3	33.72	298.28	2.98	2.18×10^5	2.18×10^3	2.01×10^1
Operator 2	Test 4	32.11	281.04	3.20	2.23×10^5	2.08×10^3	2.21×10^1
	Test 5	33.34	280.67	2.73	2.28×10^5	2.08×10^3	2.21×10^1
	Test 6	29.33	281.80	2.62	2.17×10^5	2.06×10^3	1.95×10^1
Operator 3	Test 7	34.75	311.24	2.97	2.25×10^5	2.13×10^3	1.85×10^1
	Test 8	35.32	308.51	2.96	2.26×10^5	2.04×10^3	2.07×10^1
	Test 9	35.30	326.13	3.32	2.33×10^5	2.1×10^3	2.16×10^1
/	Mean	33.84	298.85	3.03	2.25×10^5	2.11×10^3	2.06×10^1
/	CV	6.02%	5.21%	7.98%	2.27%	2.56%	5.92%

4. Specificity

4.1 Interference Test

Genomic DNA from commonly used cell lines in biopharmaceutical production (E. coli, CHO, Hansenula, Vero) was tested for interference with the Sf9 and baculovirus AcNPV residual DNA detection assay. Specifically, 3 pg of E. coli, CHO, Hansenula, and Vero genomic DNA were used as templates. Results showed no amplification in either FAM or VIC channels, indicating no significant interference.

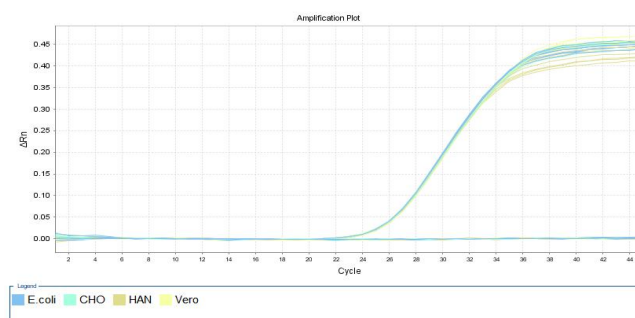


Figure 3. Interference Test Results

5. Limit of Quantitation (LOQ)

Sf9 DNA at concentrations of 3 fg/μL, 1 fg/μL, and 0.5 fg/μL, and AcNPV baculovirus DNA at 2.12×10¹ copies/μL, 7 copies/μL, and 3.5 copies/μL were tested, with 10 replicates per concentration. Results showed that for Sf9 DNA, CV < 20% at 1 fg/μL and above; for AcNPV baculovirus DNA, CV < 20% at 7 copies/μL and above. Therefore, the LOQ for Sf9 in the kit is 1 fg/μL, and for AcNPV, it is 7 copies/μL.

Table 5. LOQ Test Results for Sf9 and AcNPV DNA

Test Item Repetition	Sf9 DNA (fg/μL)		AcNPV (copies/μL)	
	Quantity	Back-calculated Ratio	Quantity	Back-calculated Ratio
1	0.81	81%	5.11	73.00%
2	0.84	84%	5.22	74.57%
3	0.91	91%	8.48	121.14%
4	0.92	92%	7.04	100.57%
5	0.78	78%	7.21	103.00%
6	1.12	112%	6.76	96.57%
7	0.71	71%	6.24	89.14%
8	1.02	102%	7.06	100.86%
9	1.06	106%	6.62	94.57%
10	0.78	78%	6.64	94.86%
Mean	0.90	\	6.64	\
CV	15.06%	\	14.70%	\

6. Durability

The kit was tested and found suitable for, but not limited to, the following instruments:

Table 6. Instrument Compatibility Test Results

Brand	Model	Gene	Amplification Efficiency	R ²	LOQ Concentration	CV
Thermo	ABI 7500	Sf9	100.78%	1	1 fg/μL	15.73%
		AcNPV	101.66%	1	7 copies/μL	14.12%
	ABI Quant Studio 5	Sf9	100.03%	1	1 fg/μL	15.60%
		AcNPV	99.01%	1	7 copies/μL	11.03%
Bio-Rad	CFX96	Sf9	99.83%	0.99	1 fg/μL	19.21%
		AcNPV	100.10%	1	7 copies/μL	14.09%
Shanghai Holmes	SLAN-96S	Sf9	104.35%	0.99	1 fg/μL	20.02%
		AcNPV	102.54%	0.99	7 copies/μL	13.33%
Bioer	LineGene9600	Sf9	103.81%	0.99	1 fg/μL	13.54%
		AcNPV	101.82%	0.99	7 copies/μL	13.81%

7. Stability

7.1 Freeze-Thaw Stability

The Sf9 and AcNPV DNA Residue Detection Kit was tested after 10 freeze-thaw cycles, and kit performance was unaffected.

Table 7. Freeze-Thaw Stability Analysis Results

Test Item	Freeze-Thaw Cycles	Test Parameter	T0 (Before)		After 10 Cycles	
			Sf9	AcNPV	Sf9	AcNPV
Standard Curve Parameters		Amplification Efficiency	101.71%	101.12%	100.26%	100.40%
		R ²	1	1	1	1
LOQ Concentration (Sf9 1 fg/μL, AcNPV 7 copies/μL)		CV	10.08%	13.7%	14.06%	10.6%

7.2 Accelerated Stability

The Sf9 and AcNPV DNA Residue Detection Kit was stored at 2–8°C for 30 days and at 37°C for 14 days. Kit performance was unaffected in both conditions.

Table 8. Accelerated Stability Analysis Results

Test Item	Accelerated Conditions	Test Parameter	TO (Before)		2–8°C 30 days		T0 (Before)		37°C 14 days	
			Sf9	AcNPV	Sf9	AcNPV	Sf9	AcNPV	Sf9	AcNPV
Standard Curve Parameters		Amplification Efficiency	101.7%	100.6%	101.7%	100.9%	100.16%	98.56%	101.85%	98.28%
		R ²	0.99	1	0.99	1	1	1	1	1
LOQ Concentration (Sf9 1 fg/μL, AcNPV 7 copies/μL)		CV	16.83%	16.08%	11.4%	8.5%	15.8%	9.7%	12.25%	19.37%

8. Limit of Blank (LOB)

8.1 No Template Control (NTC)

DNA diluent was used as sample for qPCR quantification. No amplification was observed in 48 replicate wells.

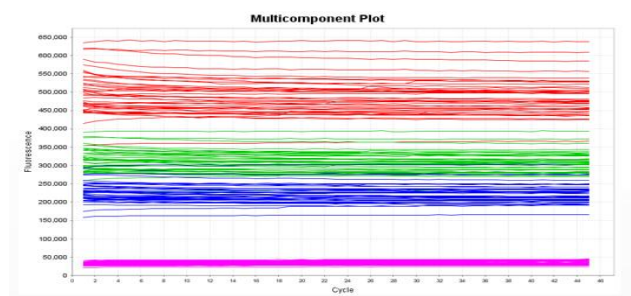


Figure 4. NTC Test Results

8.2 Negative Extraction Control (NCS)

DNA diluent was first subjected to sample pretreatment extraction, then tested by qPCR. No amplification was observed in 48 replicate wells.

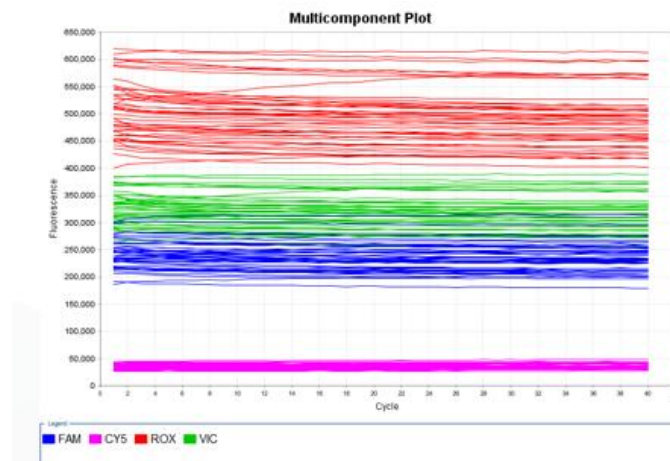


Figure 5. NCS Test Results

References

1. Chinese Pharmacopoeia Commission (ChPC). Chinese Pharmacopoeia, Volume III: Guidance on Validation of Quantitative Analytical Methods for Biological Samples [S]. Beijing: China Science and Technology Press, 2020: 542–543.
2. NMPA, 2007: General Principles for Technical Review of Analytical Method Validation in Biologics Quality Control.
3. ICH (2022) Analytical Method Validation Q2, draft version.