

Performance Verification Summary for Plasmid DNA Residue Detection Kit (Catalog No.: 41323ES)

Background

The Plasmid DNA Residue Detection Kit is an in-house developed solution by Yeasen Biotechnology (Shanghai) Co., Ltd., designed for the quantitative analysis of plasmid DNA residues in intermediates, bulk products, and finished goods across various biological products. Utilizing probe-based real-time quantitative PCR (qPCR) technology, this kit enables specific and rapid detection of residual plasmid DNA, with a limit of quantification (LOQ) as low as 4 copies/ μ L.

The data in this summary report is provided by Yeasen Biotechnology (Shanghai) Co., Ltd. Validation parameters include linearity, range, accuracy, limit of quantification, precision, robustness, and specificity. This summary data is for reference only; users are required to perform method validation for plasmid DNA residue detection according to applicable regulations and pharmacopoeia requirements, based on their specific experimental environment, conditions, and samples.

Materials and Methods

1. **Plasmid DNA Residue Detection Kit:** Cat# 41323ES60 (YEASEN)
2. **Magnetic Bead Sample Preparation Kit:** Cat# 18461ES (YEASEN)
3. **Raw Data:** Electronic records are stored in the sub-file "Product Experimental Report" under the parent file "Plasmid DNA Residue Detection Kit".
4. **Method:** The materials and operational procedures required for the experiments are primarily produced or established by Yeasen Biotechnology (Shanghai) Co., Ltd. The research, development, and manufacturing processes strictly adhere to the ISO13485 system requirements, and the performance meets the relevant requirements of ICH Q2(R1) and the 2020 Edition of the Chinese Pharmacopoeia, Volume III, Guideline 9101 for Validation of Analytical Procedures.

Validation Parameters and Results

1. Detection Range

The kit has a linear range of 4×10^1 copies/ μ L to 4×10^6 copies/ μ L, with an R^2 value of 1, amplification efficiency between 90% and 110%, and a coefficient of variation (CV) of <15% for detected values at each concentration.

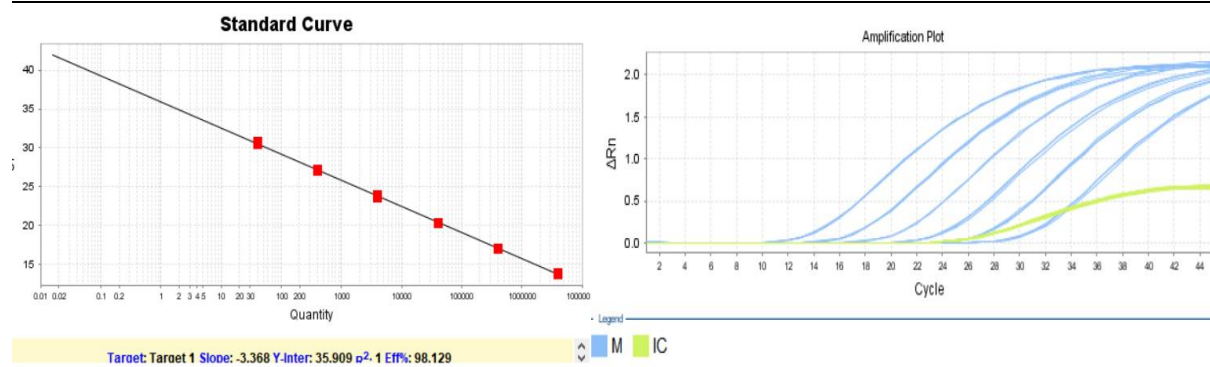


Figure 1. Plasmid DNA Standard Curve Linearity Plot (Left) and Amplification Curve Linear Profile (Right)

2. Accuracy

2.1 Recovery

2.1.1 Blank Sample Spike Recovery Experiment

Three concentrations of plasmid DNA samples (4×10^5 copies/ μ L, 4×10^3 copies/ μ L, and 4×10^1 copies/ μ L) were tested in triplicate to analyze sample recovery rates and CV. For DNA samples at different concentrations, the recovery rates were all between 70% and 130%, with CVs < 20%.

Table 1. Blank Sample Spike Recovery Rate

Sample Type	Theoretical Conc. (copies/ μ L)	Extraction 1 Mean (copies/ μ L)	Extraction 2 Mean (copies/ μ L)	Extraction 3 Mean (copies/ μ L)	Avg. Measured Conc. (copies/ μ L)	CV	Recovery
Blank Sample	/	/	/	/	/	/	/
Recovery Sample 1	4×10^5	3.88×10^5	3.69×10^5	3.76×10^5	3.79×10^5	2.56%	94.75%
Recovery Sample 2	4×10^3	3.99×10^3	4.15×10^3	4.37×10^3	4.17×10^3	4.61%	115.25%
Recovery Sample 3	4×10^1	4.01×10^1	4.28×10^1	4.35×10^1	4.22×10^1	4.06%	105.38%

2.1.2 Simulated Sample Recovery Experiment

The same concentration of sample (4×10^2 copies/ μ L) was extracted using four different background solutions (high protein, high salt, high pH, and low pH). Each sample was tested in triplicate to analyze the recovery rate and CV. For DNA samples in different background solutions, the recovery rates were all between 70% and 130%, with CVs < 20%.

Table 2. Simulated Sample Spike Recovery Rate

Sample Type	Theoretical Conc.	Replicate 1	Replicate 2	Replicate 3	Mean	CV	Recovery
Base Sample	/	/	/	/	/	/	/
High Protein	4×10^2 copies/ μ L	4.09×10^2	4.21×10^2	4.02×10^2	4.11×10^2	2.34%	103%
High Salt		3.94×10^2	3.85×10^2	3.82×10^2	3.87×10^2	1.69%	97%
High pH		4.12×10^2	3.76×10^2	4.21×10^2	4.03×10^2	5.86%	101%

Low pH		3.80×10^2	4.01×10^2	3.77×10^2	3.86×10^2	3.32%	96%
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3. Precision

3.1 Repeatability

Plasmid DNA at a concentration of 4×10^1 copies/ μ L (i.e., 40 copies/ μ L) was tested 10 times consecutively. The CV was < 15%, meeting the repeatability requirements.

Table 3. Repeatability Experimental Results

Replicate Number	1	2	3	4	5	6	7	8	9	10	Mean	CV
Measured Value (copies/ μ L)	43.44	52.58	47.47	49.16	51.7	48.67	37.36	36.52	43.81	39.94	45.07	12.79%

3.2 Intermediate Precision

Three operators independently tested plasmid DNA at concentrations of 4×10^5 copies/ μ L, 4×10^3 copies/ μ L, and 4×10^1 copies/ μ L. Each concentration was tested in triplicate, and the CV for the resulting 9 data points was < 15%.

Table 4. Intermediate Precision Test Results

Operator	Theoretical Conc. Measured Conc.	Plasmid DNA(Copies/ μ L)		
		4×10^5	4×10^3	4×10^1
Operator 1	Measurement 1	3.80×10^5	4.56×10^3	3.60×10^1
	Measurement 2	4.09×10^5	4.54×10^3	3.25×10^1
	Measurement 3	3.83×10^5	4.50×10^3	3.44×10^1
Operator 2	Measurement 4	3.74×10^5	4.08×10^3	3.46×10^1
	Measurement 5	3.87×10^5	4.38×10^3	3.51×10^1
	Measurement 6	3.76×10^5	4.40×10^3	3.44×10^1
Operator 3	Measurement 7	3.79×10^5	4.26×10^3	3.95×10^1
	Measurement 8	3.55×10^5	4.22×10^3	3.54×10^1
	Measurement 9	3.70×10^5	3.71×10^3	3.21×10^1
/	Mean	3.80×10^5	4.37×10^3	3.49×10^1
/	CV	3.80%	6.28%	6.14%

4. Specificity

4.1 Interference Experiment

The interference of genomic DNA from common engineering host cells used in biological product manufacturing was evaluated. The test values for the interference groups were all significantly lower than the negative control, showing no obvious interference.

Table 5. Interference Experiment Results

Cell Line	Mean Detection (copies/ μ L)	Comparison with NTC Value
NTC(DNA Diluent)	0.95	/
Vero	0.33	<NTC
Sf9	0.21	<NTC
HEK293	0.41	<NTC
CHO	0.14	<NTC

5. Limit of Quantification (LOQ)

Plasmid DNA at concentrations of 40 copies/ μ L, 10 copies/ μ L, 4 copies/ μ L, and 1 copy/ μ L was tested, with 10 replicates for each concentration. Results showed that at concentrations of 4 copies/ μ L and above, the CV was < 20%. This indicates that the Limit of Quantification (LOQ) of the Plasmid DNA Residue Detection Kit is 4 copies/ μ L.

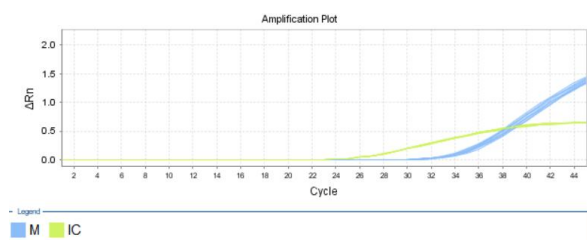


Figure 2. qPCR Detection Results for 4 copies/ μ L Plasmid DNA

Items Replicate	Plasmid DNA (copies/ μ L)	
	Quantity	Back-calculated Ratio
1	5.33	133.15%
2	4.14	103.45%
3	5.54	138.44%
4	4.47	111.77%
5	5.85	146.27%
6	4.94	123.59%
7	3.63	90.85%
8	4.3	107.49%
9	4.1	102.39%
10	4.37	109.19%
Mean	4.67	\
CV	15.33%	\

Table 6. Plasmid DNA Limit of Quantification (LOQ) Test Results

6. Robustness

Testing has demonstrated that this kit is compatible with, but not limited to, the following instruments:

Table 7. Instrument Compatibility Test Results

Brand	Model	Amplification Efficiency	R ²	LOQ Conc. (copies/μL)	CV
Thermo	ABI 7500	98.13%	1	4	15.33%
Thermo	ABI Quant Studio 5	97.86%	1	4	18.54%
Shanghai Hongshi	SLAN-96S	101.90%	0.999	4	18.66%

7. Stability

7.1 Freeze-Thaw Stability

The Plasmid DNA Residue Detection Kit was subjected to 10 freeze-thaw cycles at -20°C. The results showed that the kit performance was not affected.

Table 8. Analysis of Freeze-Thaw Stability Results

Freeze-Thaw Cycles			
Test Items	Parameters	T0 Time Point	After 10 Freeze-Thaw Cycles
Standard Curve Parameters	Amplification Efficiency	97.58%	97.66%
	R ²	1	1
LOQ Conc. (4 copies/μL)	CV	18.82%	17.24%

7.2 Accelerated Stability

The Plasmid DNA Residue Detection Kit was stored at 2~8°C for 30 days and at 37°C for 14 days. The kit performance remained unaffected under both conditions.

Table 9. Accelerated Stability Result Analysis

Accelerated condition		Experiment 1		Experiment 2	
Test Item	Parameters	T0 Time Point	2~8°C for 30 days	T0 Time Point	37°C for 14days
Standard Curve Parameters	Amplification				
	Efficiency	96.86%	100.49%	99.37%	99.30%
	R ²	1	1	1	1
LOQ Conc. (4 copies/μL)	CV	16.84%	13.74%	12.91%	18.60%

8. Limit of Blank (LOB)

8.1 No Template Control (NTC)

Using DNA diluent as the sample for qPCR quantitative detection, the Ct values for over 48 replicate wells were all > 37, meeting the LOB requirements.

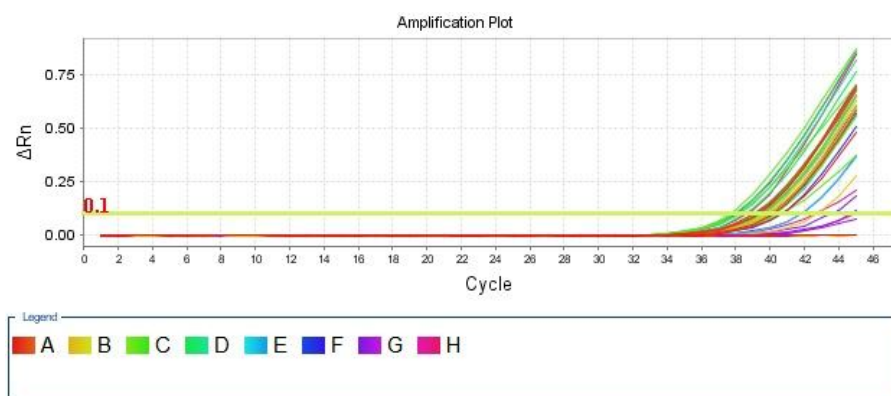


Figure 3. NTC Experimental Detection Results

8.2 Negative Control Sample (NCS)

Using DNA diluent as the sample, which was first subjected to sample preparation extraction and then qPCR detection, the Ct values for 48 replicate wells were all > 36, meeting the LOB requirements.

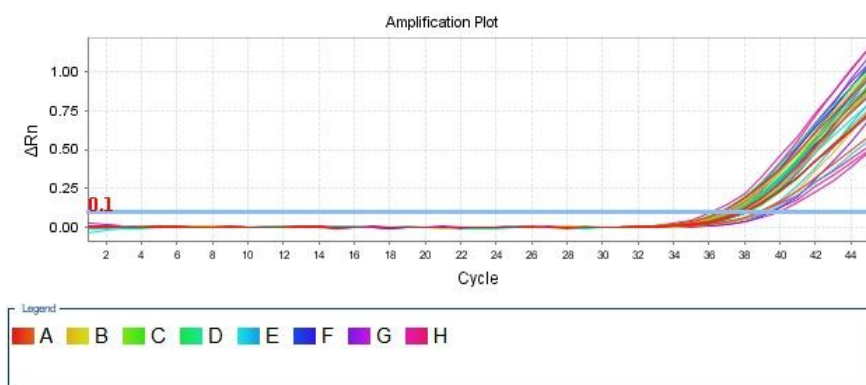


Figure 4. NCS Experimental Detection Results

References

1. Chinese Pharmacopoeia Commission (ChPC). Chinese Pharmacopoeia Volume III: Guideline for Validation of 2. Quantitative Analytical Procedures for Biological Samples [S]. Beijing: China Science and Technology Press, 2020: 542-543.
2. NMPA, 2007: General Principles for Technical Review of Method Validation for Quality Control of Biological Products.
3. ICH (2022) Analytical method validation Q2, draft version.