

HEK293 Host Cell Residue DNA Size Analysis Kit

Performance Validation Summary (Catalog No.: 41316ES70)

Background

The HEK293 Host Cell Residue DNA Size Analysis Kit is an in-house developed product by Yeasen Biotechnology (Shanghai) Co., Ltd. It is designed to analyze the size distribution of residual host cell DNA derived from HEK293 cells in intermediates, semi-finished products, and final biologics produced using HEK293 expression systems.

This kit employs a fluorescence probe-based qPCR method and includes four distinct amplicon targets to quantitatively assess residual HEK293 DNA fragments of varying lengths in samples.

The data presented in this validation summary were generated by Yeasen Biotechnology (Shanghai) Co., Ltd. Validated parameters include linearity, range, accuracy, precision, specificity, limit of quantitation (LOQ), robustness, stability, and limit of blank (LOB). This summary is for reference only. Users must perform their own method validation according to relevant pharmacopoeial and regulatory requirements, considering their specific experimental conditions and sample matrices.

Materials and Methods

1. HEK293 Host Cell Residue DNA Size Analysis Kit: Cat# 41316ES70 (YEASEN)
2. Magnetic Bead-Based Residual DNA Sample Preparation Kit: Cat# 18461ES (YEASEN)
3. Raw Data: Electronically recorded in the sub-document Product Experimental Report under the master file HEK293 Residual DNA Fragment Analysis Kit.
4. Methodology: All materials and protocols were developed and manufactured by Yeasen Biotechnology (Shanghai) Co., Ltd. in compliance with ISO 13485 quality management system requirements. Performance meets the criteria outlined in ICH Q2(R1) and the Chinese Pharmacopoeia (ChP) 2020, Volume III, Guideline 9101: Validation of Analytical Procedures.

Validation Content and Results

1. Assay Range

The kit contains four amplicon targets of different lengths: 82 bp, 133 bp, 227 bp, and 515 bp. A standard curve must be established separately for each fragment.

For all four fragments:

Linear range: 30 fg/μL – 300 pg/μL

$R^2 > 0.99$

Amplification efficiency: 90% – 110%

CV of measured concentrations across all levels: <15%

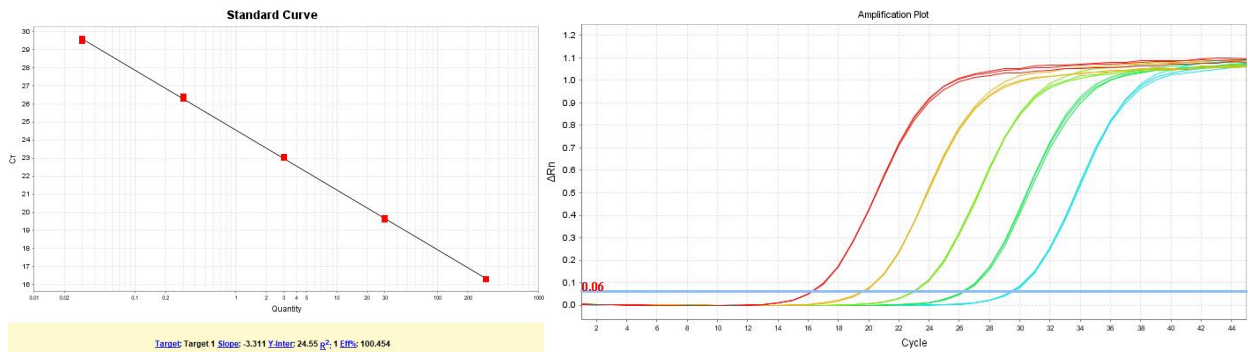


Figure 1. Standard curve linearity plot (left) and amplification curve profile (right) for the 82 bp amplicon.

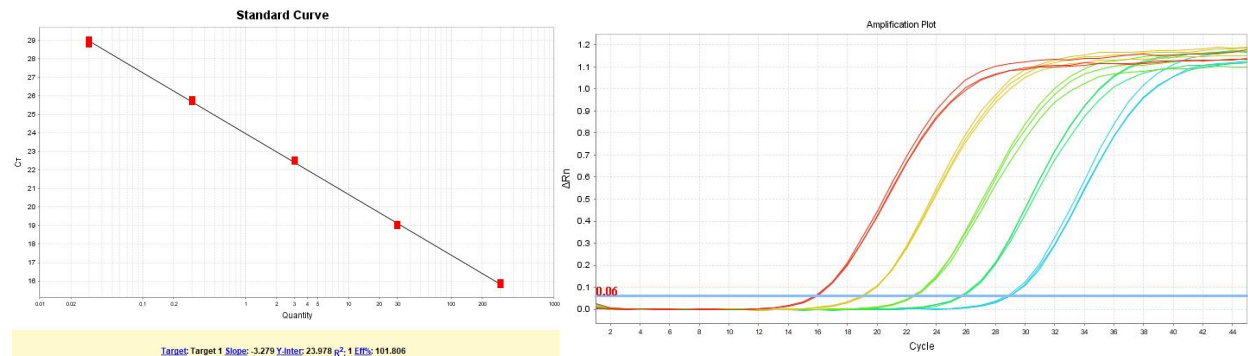


Figure 2. Standard curve linearity plot (left) and amplification curve profile (right) for the 133 bp amplicon.

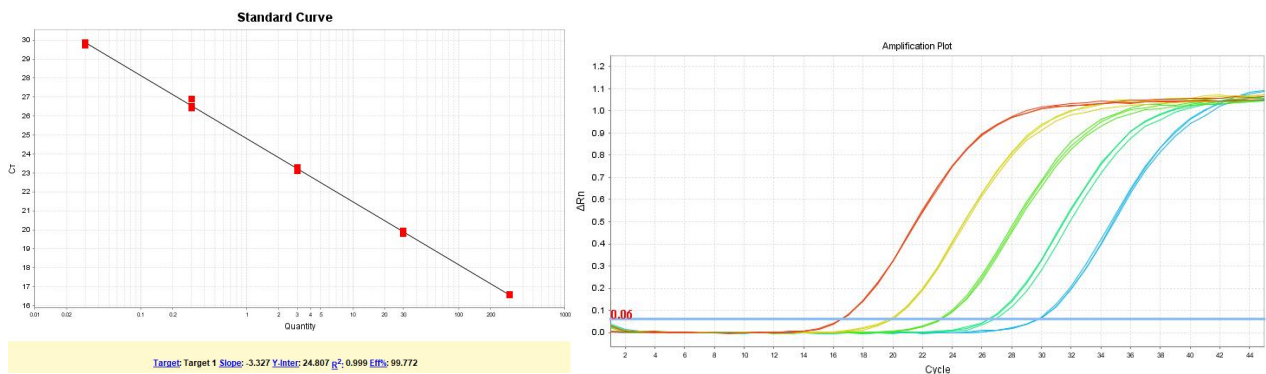


Figure 3. Standard curve linearity plot (left) and amplification curve profile (right) for the 227 bp amplicon.

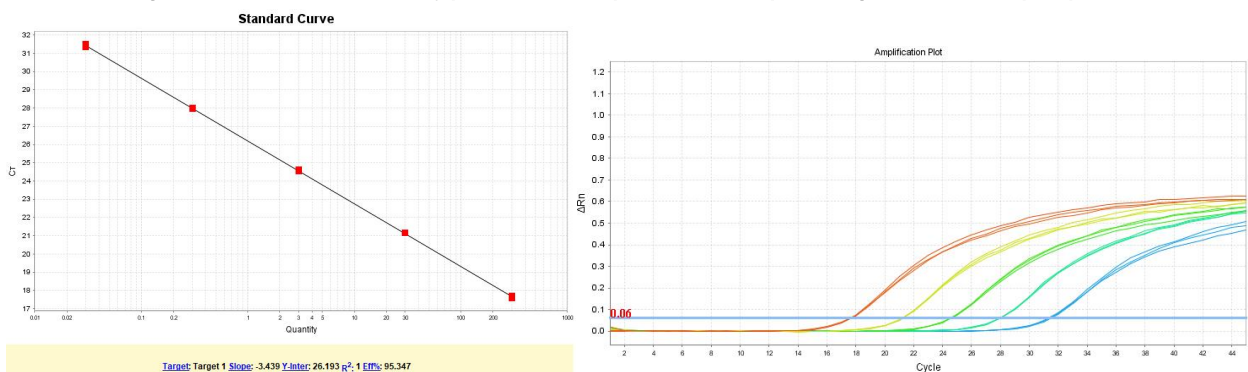


Figure 4. Standard curve linearity plot (left) and amplification curve profile (right) for the 515 bp amplicon.

2. Accuracy

2.1 Recovery

2.1.1 Spiked Recovery in Blank Matrix

HEK293 DNA samples at high, medium, and low concentrations—300 pg/μL, 3 pg/μL, and 30 fg/μL (i.e., 0.03 pg/μL)—were prepared in a blank matrix. Samples were extracted using the Magnetic Bead-Based Residual DNA Sample Preparation Kit (3 extraction replicates per sample, each with 3 technical replicates).

Recovery for all concentrations ranged from 70% to 130%, with CV < 20%.

Table 1. Spiked Recovery in Blank Matrix

Fragment (bp)	Sample Type	Theoretical Concentration (pg/μL)	Extract 1 Mean (pg/μL)	Extract 2 Mean (pg/μL)	Extract 3 Mean (pg/μL)	Average Measured Concentration (pg/μL)	CV	Recovery
82 bp	Blank Sample	/	/	/	/	/	/	/
	Spiked Sample 1	300	306.811	279.569	295.082	293.821	5%	98%
	Spiked Sample 2	3	3.113	2.998	3.168	3.093	3%	103%
	Spiked Sample 3	0.03	0.044	0.035	0.048	0.042	15%	141%
133 bp	Blank Sample	/	/	/	/	/	/	/
	Spiked Sample 1	300	353.865	335.931	324.028	337.941	4%	113%
	Spiked Sample 2	3	3.491	3.361	3.124	3.325	6%	111%
	Spiked Sample 3	0.03	0.038	0.034	0.045	0.039	14%	130%
227 bp	Blank Sample	/	/	/	/	/	/	/
	Spiked Sample 1	300	266.957	279.678	274.549	273.728	2%	91%
	Spiked Sample 2	3	2.609	2.608	2.709	2.642	2%	88%
	Spiked Sample 3	0.03	0.035	0.037	0.035	0.036	4%	119%
515 bp	Blank Sample	/	/	/	/	/	/	/
	Spiked Sample 1	300	283.646	300.483	291.244	291.791	3%	97%

Fragment (bp)	Sample Type	Theoretical Concentration (pg/μL)	Extract 1 Mean (pg/μL)	Extract 2 Mean (pg/μL)	Extract 3 Mean (pg/μL)	Average Measured Concentration (pg/μL)	CV	Recovery
	Spiked Sample 2	3	2.780	2.826	2.997	2.868	4%	96%
	Spiked Sample 3	0.03	0.037	0.037	0.038	0.037	1%	124%

2.1.2 Spiked Recovery in Simulated Matrices

A fixed concentration of HEK293 DNA (3 pg/μL) was spiked into five different background solutions: high protein, high nucleic acid, high salt, high pH, and low pH. Each condition underwent 3 extraction replicates, each with 3 technical replicates.

Recovery across all matrices ranged from 70% to 130%, with CV < 20%.

Table 2. Spiked Recovery in Simulated Matrices

Fragment (bp)	Sample Type	Theoretical Concentration (pg/μL)	Extract 1 Mean (pg/μL)	Extract 2 Mean (pg/μL)	Extract 3 Mean (pg/μL)	Average Measured Concentration (pg/μL)	CV	Recovery
82 bp	Baseline Sample	/	/	/	/	/	/	/
	High Protein	3	2.746	2.818	2.834	2.800	2%	93%
	High Nucleic Acid		3.047	3.058	2.815	2.973	5%	99%
	High Salt		2.835	2.904	2.886	2.875	1%	96%
	High pH		2.866	2.939	2.990	2.932	2%	98%
	Low pH		2.817	2.759	2.805	2.794	1%	93%
133 bp	Baseline Sample	/	/	/	/	/	/	/
	High Protein	3	2.261	2.386	2.484	2.377	5%	79%
	High Nucleic Acid		2.764	2.836	2.753	2.785	2%	93%
	High Salt		2.438	2.850	2.715	2.668	8%	89%
	High pH		2.584	2.795	2.692	2.690	4%	90%
	Low pH		2.724	2.526	2.315	2.522	8%	84%
227 bp	Baseline	/	/	/	/	/	/	/

Fragment (bp)	Sample Type	Theoretical Concentration (pg/μL)	Extract 1 Mean (pg/μL)	Extract 2 Mean (pg/μL)	Extract 3 Mean (pg/μL)	Average Measured Concentration (pg/μL)	CV	Recovery
	Sample							
	High Protein	3	3.062	2.736	3.096	2.965	7%	99%
	High Nucleic Acid		2.964	2.882	3.035	2.960	3%	99%
	High Salt		2.783	2.692	3.100	2.858	7%	95%
	High pH		2.814	2.909	3.036	2.920	4%	97%
	Low pH		2.919	2.651	3.232	2.934	10%	98%
515 bp	Baseline Sample	/	/	/	/	/	/	/
	High Protein	3	2.561	2.555	2.977	2.698	9%	90%
	High Nucleic Acid		2.776	2.596	3.050	2.808	8%	94%
	High Salt		2.330	2.624	2.864	2.602	10%	87%
	High pH		2.416	2.749	2.807	2.657	8%	89%
	Low pH		2.898	2.560	2.702	2.750	6%	92%

3. Precision

3.1 Repeatability

Each of the four primer-probe sets (82 bp, 133 bp, 227 bp, 515 bp) was used to test the lowest standard curve concentration (30 fg/μL HEK293 DNA) in 10 replicates. CV < 15% for all fragments.

Table 3. Repeatability Results

Fragment (bp)	Replicate	1	2	3	4	5	6	7	8	9	10	Mean (fg/μL)	CV (%)
82	Measured Value (fg/μL)	29.78	30.12	30.07	30.03	27.98	31.01	31.04	31.01	31.07	29.94	30.21	3.12
133	Measured Value (fg/μL)	29.85	29.91	30.09	30.05	31.03	33.01	32.14	33.02	32.02	32.04	31.32	4.09
227	Measured Value (fg/μL)	25.9	26.8	25.95	26.77	29.31	28.8	31.01	27.9	30.0	30.0	28.28	6.47

Fragment (bp)	Replicate	1	2	3	4	5	6	7	8	9	10	Mean (fg/ μ L)	CV (%)
	Value (fg/ μ L)	7	8				4		9	2	6		
515	Measured Value (fg/ μ L)	31.12	30.26	33.04	30.33	29.89	32.05	30.47	32.08	32.16	31.49	31.29	3.31

3.2 Intermediate Precision

Three independent operators used the four primer-probe sets to test HEK293 DNA at 300 pg/ μ L, 3 pg/ μ L, and 30 fg/ μ L (0.03 pg/ μ L). Each concentration was tested in triplicate per operator (total n=9). CV < 15% for all conditions.

Table 4. Intermediate Precision Results

Operator	Measured Concentration	Theoretical Concentration		
		82bp		
		300pg/ μ L	3pg/ μ L	0.03pg/ μ L
Operator 1	Measurement 1	319.579	3.026	0.032
	Measurement 2	309.761	3.051	0.037
	Measurement 3	309.243	3.067	0.036
Operator 2	Measurement 4	308.339	2.909	0.029
	Measurement 5	308.544	2.900	0.029
	Measurement 6	305.834	2.921	0.028
Operator 3	Measurement 7	299.106	3.035	0.029
	Measurement 8	300.067	2.833	0.031
	Measurement 9	302.033	3.033	0.033
/	Mean	306.945	2.975	0.032
/	CV	2%	3%	10%
Operator	Measured Concentration	Theoretical Concentration		
		133bp		
		300pg/ μ L	3pg/ μ L	0.03pg/ μ L
Operator 1	Measurement 1	292.481	2.995	0.034
	Measurement 2	314.291	3.017	0.034
	Measurement 3	279.912	2.979	0.034
Operator 2	Measurement 4	306.249	2.786	0.029
	Measurement 5	295.137	2.823	0.029
	Measurement 6	309.113	2.801	0.031
Operator 3	Measurement 7	313.483	3.152	0.031
	Measurement 8	304.683	2.842	0.029
	Measurement 9	297.709	3.076	0.037
/	Mean	301.451	2.941	0.032

/	CV	4%	5%	9%
Operator	Measured Concentration	Theoretical Concentration		
		227bp		
		300pg/μL	3pg/μL	0.03pg/μL
Operator 1	Measurement 1	300.162	2.901	0.030
	Measurement 2	321.309	3.186	0.031
	Measurement 3	301.217	3.023	0.029
Operator 2	Measurement 4	354.027	3.202	0.033
	Measurement 5	357.468	2.994	0.033
	Measurement 6	335.810	3.193	0.031
Operator 3	Measurement 7	285.265	2.947	0.030
	Measurement 8	300.848	3.301	0.030
	Measurement 9	289.026	3.378	0.031
/	Mean	316.126	3.125	0.031
/	CV	9%	5%	4%
Operator	Measured Concentration	Theoretical Concentration		
		515bp		
		300pg/μL	3pg/μL	0.03pg/μL
Operator 1	Measurement 1	308.667	3.166	0.029
	Measurement 2	299.511	3.077	0.031
	Measurement 3	299.203	3.151	0.031
Operator 2	Measurement 4	316.996	2.928	0.033
	Measurement 5	335.566	3.137	0.031
	Measurement 6	297.472	2.985	0.032
Operator 3	Measurement 7	320.271	3.072	0.029
	Measurement 8	348.454	2.992	0.025
	Measurement 9	344.742	3.069	0.028
/	Mean	318.987	3.064	0.030
/	CV	6%	3%	9%

4. Specificity

The four primer-probe sets were evaluated for cross-reactivity with genomic DNA from commonly used bioproduction cell lines (CHO, *E. coli*, *Pichia pastoris*). No amplification was observed in interference groups.

Test samples: HEK293T alone; HEK293T + CHO; HEK293T + *E. coli*; HEK293T + *Pichia pastoris*.

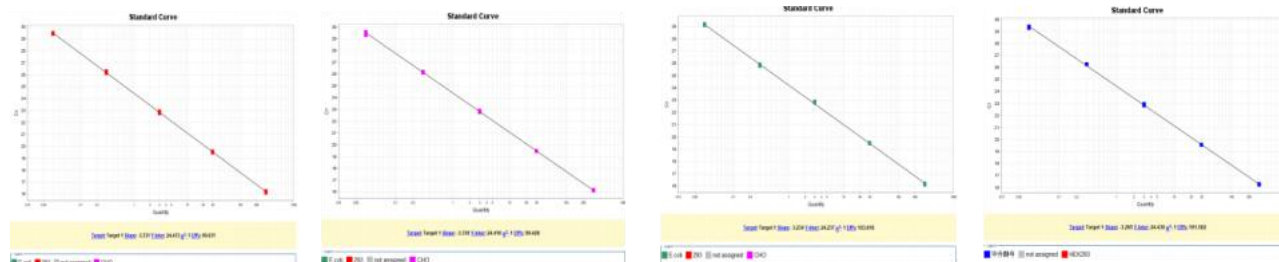


Figure 5. Interference test results for 82 bp fragment

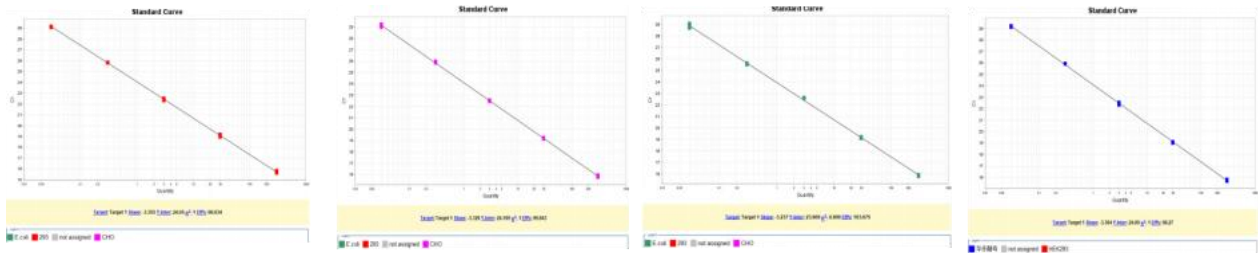


Figure 6. Interference test results for 133 bp fragment

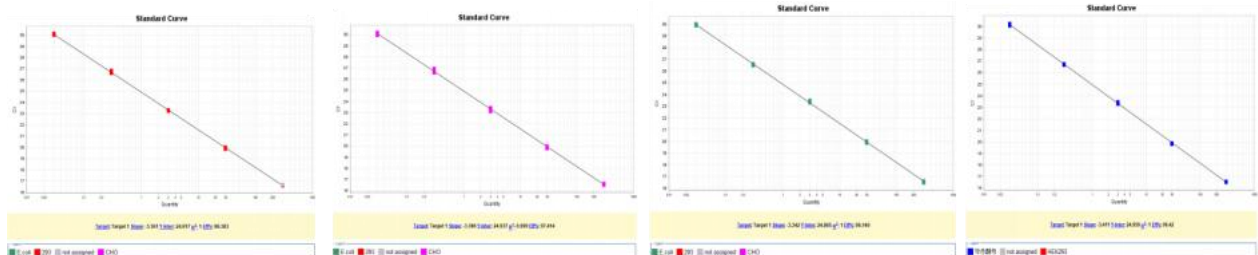


Figure 7. Interference test results for 227 bp fragment

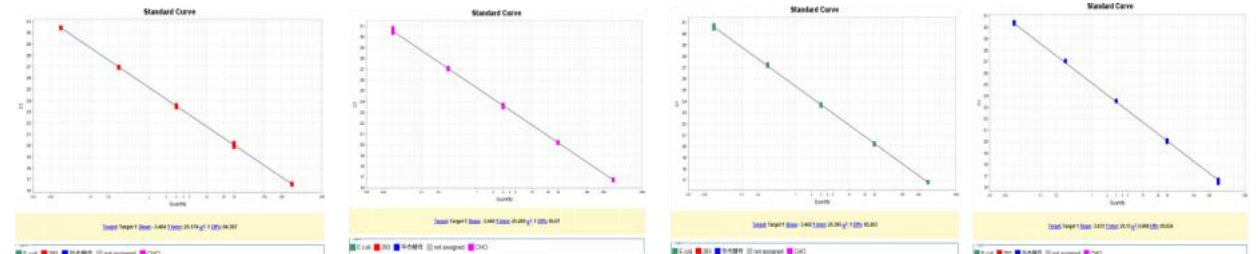


Figure 8. Interference test results for 515 bp fragment

5. Limit of Quantitation (LOQ)

HEK293 DNA at 30 fg/μL, 20 fg/μL, and 10 fg/μL (0.01 pg/μL) was tested in 10 replicates per concentration per fragment.

At ≥10 fg/μL, all four fragments showed:

CV < 20%

Recovery (back-calculated accuracy) between 70%–130%

Thus, the LOQ for all fragments is 10 fg/μL (0.01 pg/μL).

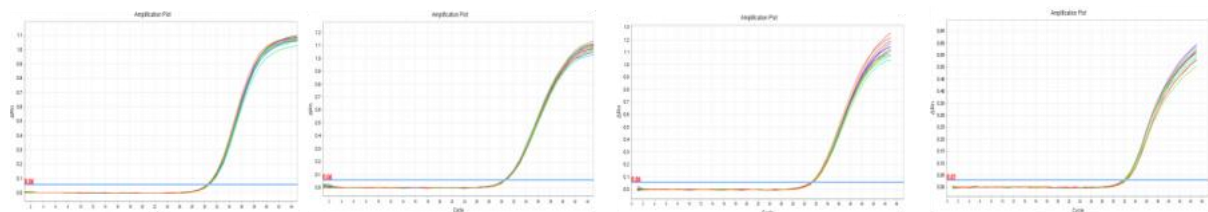


Figure 9. Amplification curves for 82 bp, 133 bp, 227 bp, and 515 bp fragments at 10 fg/μL

Table 5. LOQ Test Results (10 fg/μL)

Analyte	HEK293 DNA (fg/μL)							
	82 bp		133 bp		227 bp		515 bp	
	Quantity	Back-calculated Ratio	Quantity	Back-calculated Ratio	Quantity	Back-calculated Ratio	Quantity	Back-calculated Ratio
Replicate								

Replicate	HEK293 DNA (fg/ μ L)							
	82 bp		133 bp		227 bp		515 bp	
	Quantity	Back-calculated Ratio	Quantity	Back-calculated Ratio	Quantity	Back-calculated Ratio	Quantity	Back-calculated Ratio
1	0.011	110%	0.009	90%	0.010	95%	0.009	88%
2	0.009	91%	0.009	93%	0.009	86%	0.009	93%
3	0.011	107%	0.009	95%	0.009	87%	0.009	88%
4	0.011	112%	0.010	97%	0.009	92%	0.009	93%
5	0.011	108%	0.010	95%	0.009	87%	0.010	99%
6	0.009	94%	0.010	102%	0.009	90%	0.010	98%
7	0.010	97%	0.009	93%	0.009	87%	0.009	87%
8	0.010	100%	0.011	111%	0.010	101%	0.009	91%
9	0.010	102%	0.010	102%	0.008	83%	0.009	94%
10	0.010	100%	0.009	90%	0.008	84%	0.010	101%
Mean	0.010	\	0.010	\	0.090	\	0.008	\
CV%	7%	\	7%	\	8%	\	14%	\

6. Robustness (Instrument Compatibility)

The kit is compatible with multiple real-time PCR instruments:

Table 6. Instrument Compatibility Results

Brand	Instrument Model	Amplicon Length	Amplification Efficiency	R ²	LOQ Concentration (fg/ μ L)	CV (%)
Thermo	ABI 7500	82 bp	102.844%	1	10	7%
		133 bp	100.902%	1	10	7%
		227 bp	100.139%	0.999	10	8%
		515 bp	97.438%	1	10	14%
Thermo	ABI Q5	82 bp	104.696%	0.999	10	9%
		133 bp	101.333%	0.999	10	7%
		227 bp	101.827%	1	10	13%
		515 bp	95.260%	0.999	10	12%
Hongshi	SLAN-96S	82 bp	101.461%	0.999	10	8%
		133 bp	99.535%	0.999	10	11%
		227 bp	97.067%	0.999	10	14%
		515 bp	95.614%	0.999	10	10%

7. Stability

7.1 Freeze-Thaw Stability

After 10 freeze-thaw cycles, kit performance remained unaffected.

Table 7. Freeze-Thaw Stability Results

Freeze-Thaw Analyte	Parameter	82 bp		133 bp		227 bp		515 bp	
		T0	10 Cycles	T0	10 Cycles	T0	10 Cycles	T0	10 Cycles
Standard Curve Parameters	Amplification Efficiency (%)	99.342	100.783	100.387	102.489	99.997	98.516	94.208	96.42
	R ²	1	1	1	1	1	1	1	0.999
LOQ (10 fg/μL)	CV	11%	7%	6%	5%	7%	7%	10%	8%

7.2 Accelerated Stability

After storage at 37°C for 14 days, kit performance remained unaffected.

Table 8. Accelerated Stability Results

Freeze-Thaw Analyte	Parameter	82 bp		133 bp		227 bp		515 bp	
		T0	10 Cycles	T0	10 Cycles	T0	10 Cycles	T0	10 Cycles
Standard Curve Parameters	Amplification Efficiency (%)	103.832	100.466	99.354	99.851	98.461	98.342	95.393	97.027
	R ²	0.999	1	1	1	1	1	1	1
LOQ (10 fg/μL)	CV	8%	5%	7%	7%	4%	5%	8%	9%

8. Limit of Blank (LOB)

No-Template Control (NTC): DNA dilution buffer was used as sample.

82 bp, 133 bp, 227 bp: Ct > 32 in all 96 replicates

515 bp: Ct > 34 in all 96 replicates

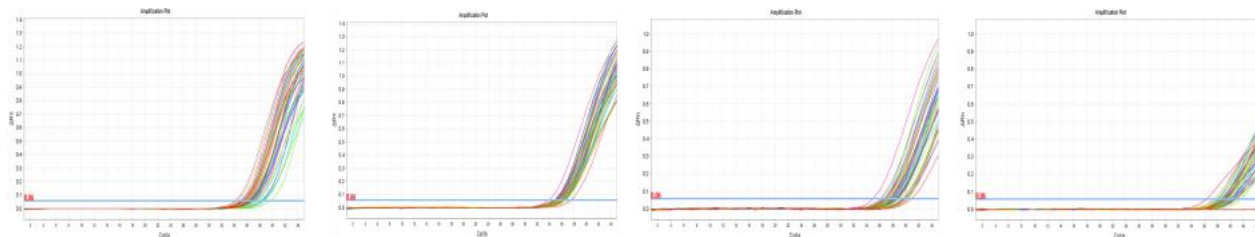


Figure 10. NTC results for 82 bp, 133 bp, 227 bp, and 515 bp fragments

References

1. Chinese Pharmacopoeia Commission (ChPC). Chinese Pharmacopoeia, Volume III: Guideline 9101 – Validation of Analytical Procedures for Quantitative Determination of Biological Samples [S]. Beijing: China Science & Technology Press, 2020: 542–543.
2. NMPA (2007). General Principles for Technical Review of Analytical Method Validation for Quality Control of Biological Products.
3. ICH (2022). Validation of Analytical Procedures: Q2(R2), Draft Version.