

SV40LTA & E1A Residue DNA Detection Kit

Performance Verification Summary (Catalog No.: 41310ES60)

I. Background

The SV40LTA & E1A Residue DNA Detection Kit is an in-house developed product by Yeasen Biotechnology (Shanghai) Co., Ltd., designed for quantitative detection of residual host cell DNA—specifically SV40 large T antigen (SV40LTA) and adenovirus E1A sequences—from biological products derived from host cells such as HEK293T. The kit employs a fluorescent probe-based qPCR method, enabling specific and rapid detection of SV40LTA and E1A residual DNA with a limit of quantitation (LOQ) as low as 4.5 copies/ μ L.

The data in this summary report were generated by Yeasen Biotechnology (Shanghai) Co., Ltd. Validation parameters include linearity, range, accuracy, precision, specificity, limit of quantitation (LOQ), robustness, stability, and limit of blank (LOB). This summary is provided for reference only. Users must perform full method validation according to their own experimental conditions, sample matrices, and regulatory/pharmacopoeial requirements (e.g., ChP, ICH Q2).

II. Materials and Methods

1. SV40LTA & E1A Residue DNA Detection Kit: Cat. No. 41310ES60 (YEASEN)
2. Magnetic Bead-Based Residual DNA Sample Preparation Kit: Cat. No. 18461ES (YEASEN)
3. Raw Data: Electronically recorded in the sub-file “Product Experimental Report” under the master file “SV40LTA & E1A Residue DNA Detection Kit”
4. Methodology: Experimental materials and protocols were primarily developed or specified by Yeasen Biotechnology (Shanghai) Co., Ltd. The R&D and manufacturing processes strictly comply with ISO 13485 requirements and meet the criteria outlined in ICH Q2(R1) and the Chinese Pharmacopoeia (ChP) 2020, Volume III, Guideline 9101: “Validation of Analytical Procedures.”

III. Validation Content and Results

1. Detection Range

SV40LTA: Linear range = 1.8×10^1 to 1.8×10^5 copies/ μ L, $R^2 = 1.000$, amplification efficiency = 100.25%, CV < 15% across all concentrations.

E1A: Linear range = 1.8×10^1 to 1.8×10^5 copies/ μ L, $R^2 = 1.000$, amplification efficiency = 100.25%, CV < 15% across all concentrations.

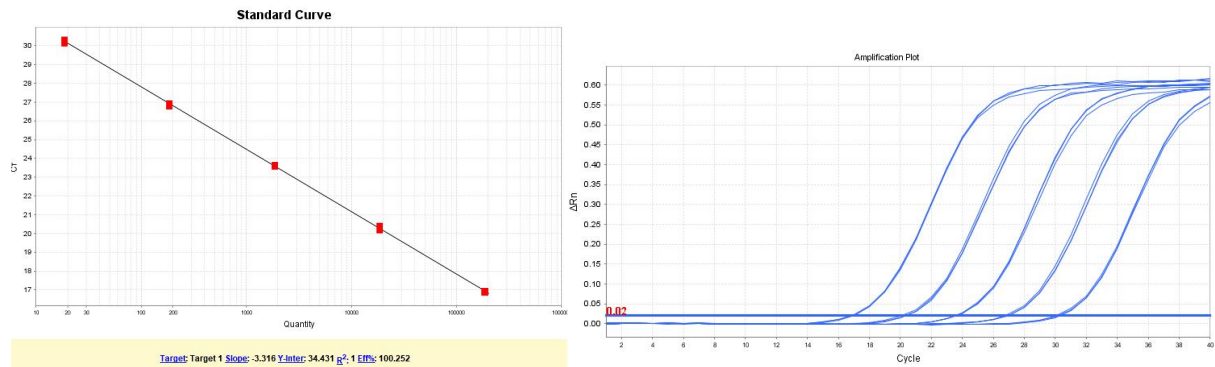


Figure 1. SV40LTA standard curve (left) and amplification plot (right)

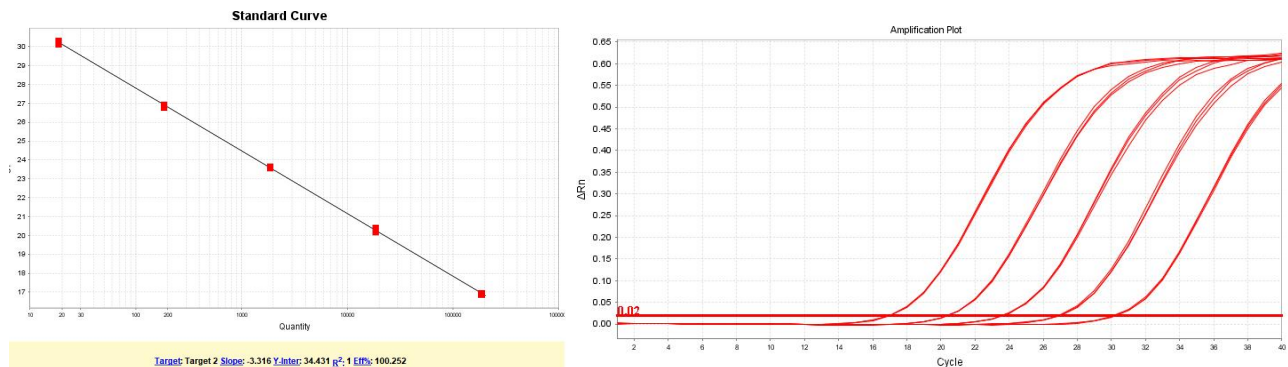


Figure 2. E1A standard curve (left) and amplification plot (right)

2. Accuracy

2.1 Recovery

2.1.1 Spiked Recovery in Blank Matrix

Samples spiked at high (1.8×10^5), medium (1.8×10^3), and low (1.8×10^1) copies/ μ L of SV40LTA and E1A DNA standards were extracted using the magnetic bead-based DNA preparation kit (two independent extractions per sample, each with three technical replicates). Recovery rates for all concentrations were within 70–130%, with CVs <20%.

Table 1. Spiked Recovery in Blank Matrix

Sample Type	Gene	Theoretical Concentration (copies/ μ L)	Mean Extraction 1 (copies/ μ L)	Mean Extraction 2 (copies/ μ L)	Mean Measured Concentration (copies/ μ L)	CV (%)	Recovery (%)
Blank	SV40LTA	—	—	—	—	—	—
	E1A	—	—	—	—	—	—
Recovery Sample 1	SV40LTA	1.8×10^5	1.53×10^5	1.55×10^5	1.54×10^5	0.17	85.77
	E1A	1.8×10^5	1.53×10^5	1.50×10^5	1.51×10^5	1.42	84.09
Recovery Sample 2	SV40LTA	1.8×10^3	1.47×10^3	1.42×10^3	1.45×10^3	2.15	80.33
	E1A	1.8×10^3	1.40×10^3	1.32×10^3	1.36×10^3	4.07	75.71
Recovery Sample 3	SV40LTA	1.8×10^1	1.89×10^1	1.54×10^1	1.72×10^1	14.31	95.33
	E1A	1.8×10^1	1.45×10^1	1.27×10^1	1.36×10^1	9.14	75.61

2.1.2 Spiked Recovery in Simulated Matrices

Five different background matrices (high protein, high nucleic acid, high salt, high pH, low pH) were spiked with SV40LTA & E1A DNA at 1.8×10^2 copies/ μ L. Each matrix underwent two extractions (each with three technical replicates). Recovery rates were within 70–130%, with CVs <20%.

Table 2. Spiked Recovery in Simulated Matrices

Sample Type	Gene	Theoretical Concentration (copies/ μ L)	Mean Extraction 1 (copies/ μ L)	Mean Extraction 2 (copies/ μ L)	Mean Measured Concentration (copies/ μ L)	CV (%)	Recovery (%)
Base Sample	SV40LTA	—	—	—	—	—	—
	E1A	—	—	—	—	—	—
High Protein	SV40LTA	1.8×10^2	1.69×10^2	1.60×10^2	1.64×10^2	3.90	91.21
	E1A		1.60×10^2	1.56×10^2	1.58×10^2	1.82	88.01
High Nucleic Acid	SV40LTA		1.98×10^2	1.80×10^2	1.89×10^2	6.60	105.02
	E1A		1.95×10^2	1.79×10^2	1.87×10^2	6.07	103.98
High Salt	SV40LTA		2.19×10^2	2.07×10^2	2.13×10^2	3.95	118.44
	E1A		2.19×10^2	2.01×10^2	2.10×10^2	6.19	116.73
High pH	SV40LTA		1.70×10^2	1.67×10^2	1.68×10^2	1.42	93.52
	E1A		1.76×10^2	1.68×10^2	1.72×10^2	3.03	95.48
Low pH	SV40LTA		1.74×10^2	1.72×10^2	1.73×10^2	0.64	96.05
	E1A		1.93×10^2	1.74×10^2	1.83×10^2	7.30	101.77

3. Precision

3.1 Repeatability

Ten replicate measurements of 1.8×10^2 copies/ μ L (i.e., 180 copies/ μ L) SV40LTA & E1A DNA standards yielded CVs <15%, meeting repeatability requirements.

Table 3. Repeatability Test Results

Replicate	1	2	3	4	5	6	7	8	9	10	Mean	CV (%)
SV40LTA (copies/ μ L)	170.75	167.03	174.14	175.16	183.79	177.50	177.14	187.85	186.38	171.60	177.14	3.90
E1A (copies/ μ L)	171.08	183.37	174.03	170.94	176.27	175.52	193.37	181.84	195.32	192.15	181.39	5.16

3.2 Intermediate Precision

Three operators independently tested high (1.8×10^5), medium (1.8×10^3), and low (1.8×10^1) concentrations of SV40LTA and E1A DNA (three replicates per concentration). All nine datasets showed CVs <15%.

Table 4. Intermediate Precision Results

Operator	Theoretical Measured	SV40 (copies/ μ L)			E1A (copies/ μ L)		
		1.8×10^5	1.8×10^3	1.8×10^1	1.8×10^5	1.8×10^3	1.8×10^1
Operator 1	Rep 1	1.83×10^5	1.81×10^3	1.63×10^1	1.82×10^5	1.79×10^3	1.99×10^1
	Rep 2	1.79×10^5	1.78×10^3	1.68×10^1	1.87×10^5	1.83×10^3	2.01×10^1
	Rep 3	1.80×10^5	1.79×10^3	1.69×10^1	1.77×10^5	1.82×10^3	1.57×10^1
Operator 2	Rep 4	1.78×10^5	1.89×10^3	1.54×10^1	1.85×10^5	1.84×10^3	1.59×10^1
	Rep 5	1.86×10^5	1.89×10^3	1.84×10^1	1.84×10^5	1.83×10^3	1.73×10^1
	Rep 6	1.75×10^5	1.81×10^3	2.17×10^1	1.83×10^5	1.80×10^3	1.68×10^1
Operator 3	Rep 7	1.79×10^5	1.81×10^3	1.59×10^1	1.89×10^5	1.76×10^3	1.60×10^1
	Rep 8	1.88×10^5	1.86×10^3	1.50×10^1	1.85×10^5	1.68×10^3	1.52×10^1
	Rep 9	1.77×10^5	1.74×10^3	1.96×10^1	1.87×10^5	1.75×10^3	2.04×10^1
/	Mean	1.81×10^5	1.82×10^3	1.74×10^1	1.84×10^5	1.79×10^3	1.75×10^1
/	CV	2.34%	2.82%	12.54%	1.88%	2.95%	11.74%

4. Specificity

4.1 Interference Study

Genomic DNA from commonly used biopharmaceutical production cell lines (Vero, CHO, *E. coli*, and human lung carcinoma NCI-H661) was evaluated for interference. No cross-reactivity or signal interference was observed—the amplification curves of interference groups overlapped with controls.

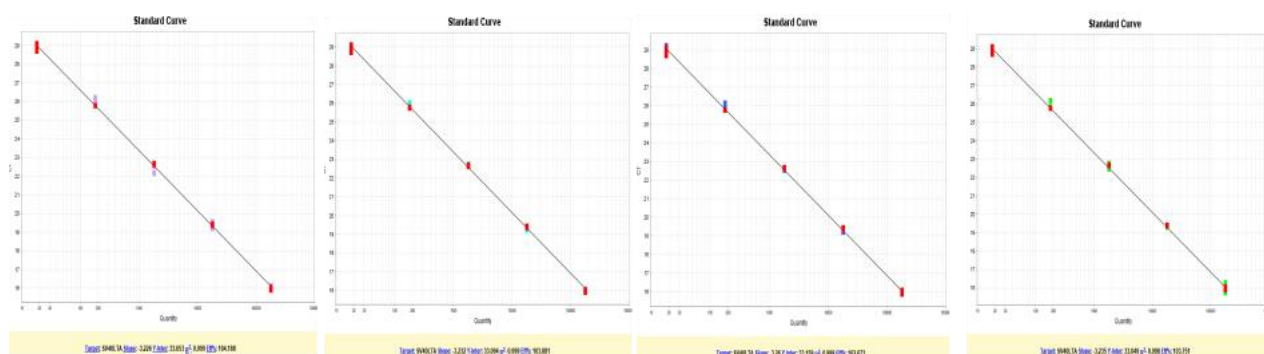


Figure 3. SV40LTA interference test results

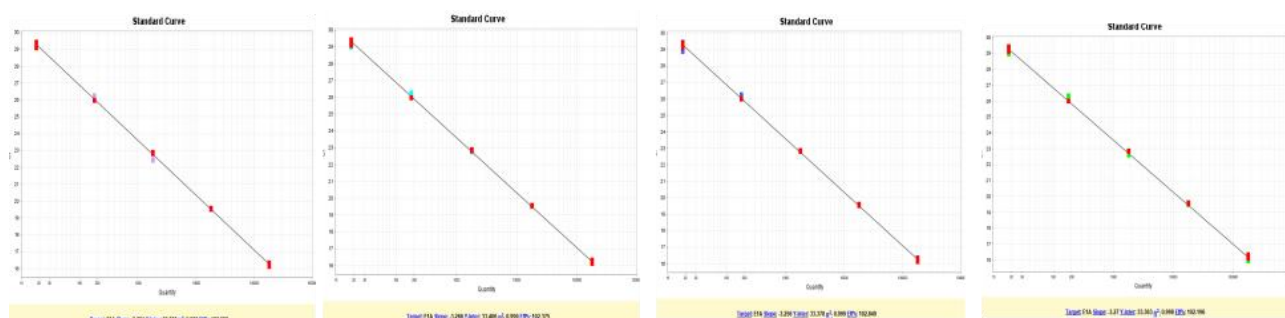


Figure 4. E1A interference test results

4.2 Detection Capability

Genomic DNA from HEK293 and HEK293T cells was tested for endogenous SV40LTA and E1A content:

HEK293: SV40LTA = 0 copies/ μ L; E1A = 371.42 copies/ μ L

HEK293T: SV40LTA = 63.00 copies/ μ L; E1A = 191.38 copies/ μ L

Results meet expected detection capability.

Table 5. Detection Capability Results

Cell Line	HEK293		HEK293T	
Gene	SV40LTA	E1A	SV40LTA	E1A
Mean (copies/ μ L)	0	371.42	63.00	191.38
CV (%)	0	0.86%	10.77%	6.39%

5. Limit of Quantitation (LOQ)

SV40LTA & E1A DNA at concentrations of 18, 9, 4.5, 2.25, and 1.8 copies/ μ L were tested in 10 replicates each. At ≥ 4.5 copies/ μ L, CVs were $<20\%$, establishing the LOQ as 4.5 copies/ μ L.

Table 6. LOQ Test Results at 4.5 copies/ μ L

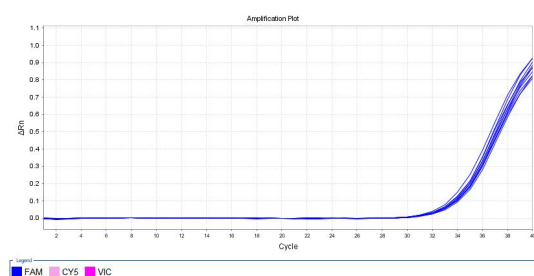


Figure 5. qPCR detection of SV40LTA at 4.5 copies/ μ L

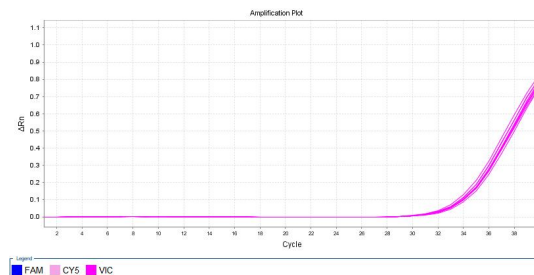


Figure 6. qPCR detection of E1A at 4.5 copies/ μ L

Replicate	SV40LTA (copies/ μ L)		E1A (copies/ μ L)	
	Quantity	Recovery	Quantity	Recovery
1	4.66	103.61%	4.87	108.21%
2	4.33	96.29%	4.15	92.26%
3	4.49	99.72%	4.63	102.88%
4	5.08	112.97%	4.86	107.99%
5	6.26	139.11%	5.67	125.93%
6	5.46	121.26%	4.97	110.42%
7	4.79	106.34%	5.50	122.22%
8	5.57	123.83%	5.00	111.20%
9	5.09	113.18%	5.55	123.28%
10	4.62	102.62%	4.97	110.43%
Mean	5.04	\	5.02	\
CV%	11.73%	\	9.13%	\

6. Robustness

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

Table 7. Instrument Compatibility Results

Brand	Model	Gene	Amplification Efficiency (%)	R ²	LOQ (copies/ μ L)	CV (%)
Thermo	ABI 7500	SV40LTA	99.07	0.999	4.5	8.03
		E1A	99.98	0.999	4.5	13.47
	QuantStudio 5	SV40LTA	98.94	0.999	4.5	7.90
		E1A	97.71	1.000	4.5	7.33
	StepOnePlus	SV40LTA	95.93	0.993	4.5	12.03
		E1A	96.30	0.999	4.5	12.12
Bio-Rad	CFX96	SV40LTA	106.51	0.996	4.5	13.28

Brand	Model	Gene	Amplification Efficiency (%)	R ²	LOQ (copies/μL)	CV (%)
		E1A	99.45	0.999	4.5	13.11
Hongshi(hanghai)	SLAN-96S	SV40LTA	98.19	0.999	4.5	11.03
		E1A	101.13	0.999	4.5	13.06

7. Stability

7.1 Non-linearized Template Stability

7.1.1 Freeze-Thaw Stability

After 10 freeze-thaw cycles, performance remained unaffected.

Table 8. Freeze-Thaw Stability (Non-linearized Standard)

Freeze-Thaw Test Parameter	Parameter	Time Zero (T ₀)		After 10 Cycles	
		SV40LTA	E1A	SV40LTA	E1A
Standard Curve Parameters	Amplification Efficiency (%)	103.41%	101.22%	102.92%	101.21%
	R ²	0.998	0.999	0.996	0.999
LOQ (4.5 copies/μL)	CV	9.09%	6.69%	3.60%	8.86%

7.1.2 Accelerated Stability

Stable after storage at 2–8°C for 30 days or 37°C for 14 days.

Table 9. Accelerated Stability (Non-linearized Standard)

Accelerated Test Parameter	Parameter	Time Zero (T ₀)		2–8°C / 30 day		Time Zero (T ₀)		37°C / 14 day	
		SV40LTA	E1A	SV40LTA	E1A	SV40LTA	E1A	SV40LTA	E1A
Standard Curve Parameters	Amplification Efficiency (%)	100.43%	98.77%	98.84%	98.38%	99.96%	98.84%	100.89%	100.17%
	R ²	1	1	0.999	1	0.999	1	0.998	0.999
LOQ (4.5 copies/μL)	CV	11.37%	9.13%	12.16%	10.30%	11.71%	9.13%	7.78%	8.43%

7.2 Linearized Template Stability

7.2.1 Freeze-Thaw Stability

Performance stable after 10 freeze-thaw cycles.

Table 10. Freeze-Thaw Stability (Linearized Standard)

Freeze-Thaw Cycles Test Parameter	Parameter	Time Zero (T ₀)		After 10 Cycles	
		SV40LTA	E1A	SV40LTA	E1A
Standard Curve Parameters	Amplification Efficiency (%)	99.18%	98.71%	100.19%	100.43%
	R ²	0.999	0.999	0.998	0.999
LOQ (4.5 copies/μL)	CV	11.73%	9.13%	19.77%	19.74%

7.2.2 Accelerated Stability

Stable under accelerated conditions.

Table 11. Accelerated Stability (Linearized Standard)

Accelerated Test	Parameter	Time Zero (T ₀)		2–8°C / 30 day		Time Zero (T ₀)		37°C / 14 day	
		SV40LTA	E1A	SV40LTA	E1A	SV40LTA	E1A	SV40LTA	E1A
Standard Curve Parameters	Amplification								
	Efficiency (%)	103.87%	104.55%	100.15%	100.74%	100.15%	100.74%	101.70%	101.40%
	R ²	1	1	1	1	1	1	1	1
LOQ (4.5 copies/μL)	CV	13.55%	9.65%	10.17%	11.63%	13.55%	9.65%	18.60%	12.99%

8. Limit of Blank (LOB)

No-template controls (NTCs) using DNA dilution buffer were run in 96 replicates. No amplification signals were detected, meeting LOB requirements.

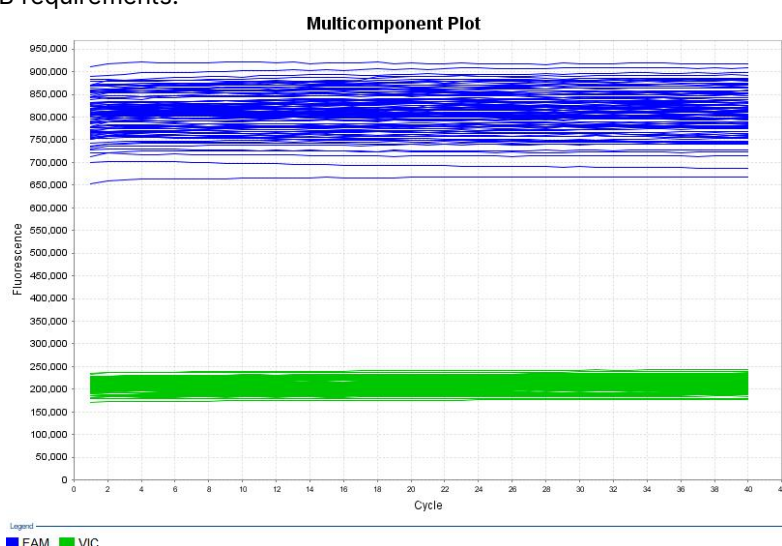


Figure 7. NTC amplification results

IV. References

1. Chinese Pharmacopoeia Commission (ChPC). Chinese Pharmacopoeia, Volume III: Guideline on 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). Analytical Procedure Validation: Q2(R2), Draft Version.