

SV40LTA&E1A Residue DNA Detection Kit

Product Description

This kit is designed for the quantitative detection of residual host cell DNA—specifically SV40 Large T Antigen (LTA) and Adenovirus E1A sequences—in biopharmaceutical products derived from host cells such as HEK293T. Utilizing probe-based real-time PCR (qPCR), the assay enables highly specific and rapid quantification of SV40 LTA and E1A residual DNA, with a limit of detection as low as 10^1 copies/ μ L.

The kit is fully compatible with Yeasen's Magnetic Bead-Based Residual DNA Sample Preparation Kits (Cat# 18461ES) for streamlined sample processing.

Specifications

Cat.No.	41310ES50 / 41310ES60
Size	50 T / 100 T

Components

Components No.	Name	41310ES50(50 T)	41310ES60(100 T)
41310-A	SV40LTA&E1A qPCR Mix	0.75 mL	1.5 mL
41310-B	SV40LTA&E1A Primer&Probe Mix	200 μ L	400 μ L
41310-C	DNA Dilution Buffer	2 $\times$ 1.8 mL	4 $\times$ 1.8 mL
41310-D	SV40LTA&E1A DNA Control (nonliner)	25 μ L	50 μ L
41310-E	SV40LTA&E1A DNA Control (linear)	25 μ L	50 μ L
41310-F	IC	50 μ L	100 μ L

*IC: Internal control.

Storage

This product should be stored at $-25\sim-15^{\circ}\text{C}$ for 2 years. Both 41310-A and 41310-B should be stored protected from light.

Applicable Instrument Models

Include but not limited to:

Bio-Rad: CFX96 Optic Module.

Thermo Scientific: ABI 7500; ABI Quant Studio 5.

Notes

1. This product is for research use only.
2. Please operate with lab coats and disposable gloves, for your safety.
3. Please read this manual carefully before using this reagent, and the experiment should be standardized, including sample handling, reaction system preparation and sample addition.
4. Ensure that each component is fully vortexized and centrifuged at low speed before use.

Instructions

I. SV40LTA&E1A DNA Standard dilution and Standard curve preparation

In this kit, the concentration of linearized and nonlinearized SV40LTA&E1A DNA Control is same. And each contains SV40LTA at a concentration of 1.8×10^7 copies/ μ L, E1A concentration is 1.8×10^7 copies/ μ L.

We recommend selecting either the linearized or non-linearized control for standard curve preparation based on the structural characteristics of your actual sample.

Standard Curve Preparation Protocol:

1) Thaw the SV40 LTA & E1A DNA Control and DNA Dilution Buffer on ice. Once fully thawed, gently vortex to mix and briefly centrifuge at low speed for 10 seconds.

2) Label six clean 1.5 mL microcentrifuge tubes as Std0, Std1, Std2, Std3, Std4, and Std5.

3) To the tube labeled Std0, add 90 μ L DNA Dilution Buffer and 10 μ L SV40 LTA & E1A DNA Control to prepare a 10-fold dilution (final concentration: $1.8 \times 1 \times 10^6$ copies/ μ L for both SV40 LTA and E1A). Vortex gently to mix, then centrifuge briefly (10 sec). This working stock (Std0) may be aliquoted and stored at -20°C for up to 3 months. Avoid repeated freeze-thaw cycles during use.

4) Add 90 μ L DNA Dilution Buffer to each of the tubes Std1 through Std5. Perform serial 10-fold dilutions as follows:

[Note]: You can continue with the dilution steps below if needed—e.g., transfer 10 μ L from Std0 to Std1, mix, then 10 μ L from Std1 to Std2, etc.—but since your original text ends here, this translation stops at step 4 as requested.

Table 1. Standard gradient dilution

Tube	Dilution Ratio	Standard concentration (copies/ μ L)	
		SV40LTA (liner/nonliner)	E1A (liner/nonliner)
Std1	10 μ L Std0 + 90 μ L DNA Dilution Buffer	1.8×10^5	1.8×10^5
Std2	10 μ L Std1 + 90 μ L DNA Dilution Buffer	1.8×10^4	1.8×10^4
Std3	10 μ L Std2 + 90 μ L DNA Dilution Buffer	1.8×10^3	1.8×10^3
Std4	10 μ L Std3 + 90 μ L DNA Dilution Buffer	1.8×10^2	1.8×10^2
Std5	10 μ L Std4 + 90 μ L DNA Dilution Buffer	1.8×10^1	1.8×10^1

[Note]:

*Three replicate wells are required for each concentration. The detection range is 1.8×10^1 copies/ μ L ~ 1.8×10^5 copies/ μ L and this range can be expanded if required.

**To reduce the number of repeat freeze-thaw and avoid contamination, it is recommended to store the DNA control in aliquots at $-25 \sim -15^\circ\text{C}$ for the first time.

***Once thawed, DNA dilution buffer could be stored at $2 \sim 8^\circ\text{C}$ for 7 days, if not used for a long time, please store at $-25 \sim -15^\circ\text{C}$.

****Make sure the template is completely mixed, gently shake the mixture for 15 secs to 1 min for each gradient dilution.

II. Preparation of Exogenous Recovery Control (ERC)

To prepare an ERC spiked with a known amount of SV40 LTA & E1A DNA (e.g., 1.8×10^3 copies of SV40 LTA and 1.8×10^3 copies of E1A), follow these steps:

1. Add 100 μ L of the test sample to a clean 1.5 mL microcentrifuge tube, then add 10 μ L of Std4. Mix thoroughly and label the tube as ERC.

2. Process the ERC alongside the batch of test samples through the same sample preparation workflow to generate the purified ERC control.

III. Preparation of Negative Control for Sample Processing (NCS)

Prepare a negative control to monitor contamination during nucleic acid extraction:

1. Add 100 μL of sample matrix (or DNA Dilution Buffer if no matrix is available) to a clean 1.5 mL microcentrifuge tube. Label it as NCS.
2. Process the NCS in parallel with the test samples using the same extraction protocol to obtain the purified NCS control.

IV. Preparation of No-Template Control (NTC)

The NTC is used to monitor reagent contamination during qPCR and is prepared at the amplification stage:

1. The NTC does not require sample extraction—it is set up directly during qPCR setup.
2. Prepare each NTC reaction (20 μL total volume) as follows:
 - 15 μL SV40 LTA & E1A qPCR Mix
 - 4 μL SV40 LTA & E1A Primer & Probe Mix
 - 1 μL Internal Control (IC)
 - 10 μL DNA Dilution Buffer (as template substitute)

It is recommended to include three replicate NTC wells/tubes per run.

V. PCR reaction system

Table 2. Reaction system

Component	Volume(μL)
SV40LTA&E1A qPCR Mix	15
SV40LTA&E1A Primer&Probe Mix	4
IC	1
DNA Template	10
Total volume	30

[Note]:

1) Calculate the total volume of Master Mix required:

Total Master Mix Volume = (Number of reaction wells + 2) $\times$ 20 μL

(The "+2" accounts for pipetting loss; each reaction contains 15 μL SV40 LTA & E1A qPCR Mix + 4 μL Primer & Probe Mix + 1 μL Internal Control [IC] = 20 μL total per well.)

Typically, each sample is tested in triplicate.

2) Determine the number of reaction wells:

Reaction Wells = (5 standard curve points + 1 NTC + 1 NCS + Number of Test Samples (TS) + Number of ERC samples) $\times$ 3

Abbreviations:

- NTC (No-Template Control): 10 μL DNA Dilution Buffer (no template added).
- NCS (Negative Control for Sample Processing): Purified extract from 100 μL sample matrix or DNA Dilution Buffer processed alongside test samples.
- TS (Test Sample): Purified DNA from your actual sample.

- ERC (Extraction Recovery Control): Purified DNA from a test sample spiked with 1.8×10^3 copies of SV40 LTA and 1.8×10^3 copies of E1A, then processed through the same extraction protocol.

3) Mixing and centrifugation after loading

After loading the reactions and sealing the tubes/plate, briefly centrifuge at low speed for 10 seconds to collect liquid from the tube/plate walls to the bottom.

Then vortex or shake for at least 5 seconds to ensure complete mixing of the reaction solution.

Centrifuge again at low speed for 10 seconds to collect liquid from the walls to the bottom of the tube/plate.

The plate layout is shown below for reference.

Table3 Computer-on reference board

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC		TS 1	TS 1	TS 1		Std 1	Std 1	Std 1			
B	NTC		TS 2	TS 2	TS 2		Std 2	Std 2	Std 2			
C	NTC		TS 3	TS 3	TS 3		Std 3	Std 3	Std 3			
D							Std 4	Std 4	Std 4			
E	NCS		ERC 1	ERC 1	ERC 1		Std 5	Std 5	Std 5			
F	NCS		ERC 2	ERC 2	ERC 2							
G	NCS		ERC 3	ERC 3	ERC 3							
H												

The plate layout includes: 5 Std (the standard curve of 5 standard concentrations), 1 NTC (no template control), 1 NCS (negative control solution), 3 TS (test samples), 3 ERC (extraction recovery control). Three replicate wells for each sample.

VI. Amplification Program Setup (Two-Step Protocol)

(Example: Applied Biosystems™ 7500 Real-Time PCR System, Software Version 2.4)

The following instructions apply only to Thermo ABI 7500 qPCR instrument (Software version 2.4). If you use a different instrument, refer to the applicable instrument guide for setup guidelines.

1. Generate a new experiment, choose the template of absolute quantification or user-defined.
2. Create two detection probe, one named "SV40LTA-DNA", select reporter fluorophore as "FAM" and quench fluorophore as "None", the other named "E1A-DNA", select reporter fluorophore as "VIC" and quench fluorophore as "None"; create 1 more detection probe, name "IC" and select the reporter fluorophore as "CY5" and quenching fluorophore as "None". The reference fluorescence is ROX" (the reference fluorescence can be based on the instrument model, etc., select whether you need to add it).
3. In the 'Assign target (s) to the selected wells' pane, set the "Task" column to "Standard", and in the "Quantity" column, assign the FAM channel "180000", "18000", "1800", "180", "18" respectively; and assign the VIC channel "180000", "18000", "1800", "180", "18" respectively; then in the corresponding "sample name" column named "Std1", "Std2", "Std3", "Std4", "Std5". Set the task of NTC as NTC. Set the NCS, TS, and ERC as Unknown, and named them according to the above Plate layout correspondingly. Then click next.

4. Set the amplification program: set the reaction volume as 30 μ L.

Table4. Amplification procedure

Cycle Step	Temperature(°C)	Time	Cycles
Initial denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	40
Annealing/Extension(Fluorescence collection)	60°C	30 sec	

VII. Analysis of qPCR results

1. The system will automatically give the Threshold In the Amplification Plot panel of Analysis. The Threshold given by the system is too close to the baseline sometimes, resulting in a large difference in Ct between replicate wells. You can manually adjust the Threshold to an appropriate position and click Analyze. Then you can initially check whether the amplification curve is normal in Multicomponent Plot.

2. In the Result Analysis tab, review the Standard Curve plot. Verify the values for the R², Efficiency, Slope and Y-intercept. For a normal standard curve, $R^2 > 0.99$, $90\% \leq \text{Eff\%} \leq 110\%$, $-3.6 \leq \text{Slope} \leq -3.1$.

3. In the 'View well table' pane in Analysis, the concentrations of each samples are showed in Quantity, the unit is copies/ μ L, the units can be convert in the assay report.

4. The parameter settings of the result analysis need to be based on the specific model and the software version used, and can generally be automatically interpreted by the instrument.

5. Calculate the spike recovery rate based on the test results of the sample TS to be measured and the sample spike recovery ERC, the recovery rate of spikes is required to be between 50%~150%.Spiked recovery rate meter formula:

Recovery (%) = {Sample spiked assay (eg.copies/ μ L) - Sample assay (eg.copies/ μ L)} x Elution volume (μ L) /Theoretical value of DNA addition amount (eg.copies) x 100%。

6. The Ct value of the negative control NCS should be greater than the mean of the lowest concentration Ct of the standard.

7. Template free control NTC should be Undetermined or Ct value ≥ 35 .