SHENTEK

Residual SV40LTA & E1A DNA Quantitation Kit (2G) User Guide

Version: A/1
For Research Use Only
Product No.: 1403443
Reagents for 100 Reactions

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(IMPORTANT: Please read this document carefully before experiment.)

1. Product information

■ Product description

SHENTEK® Residual SV40LTA & E1A DNA Quantitation Kit (2G) is used to quantitate residual SV40LTA & E1A host cell DNA, such as HEK293T cell. This kit uses duplex real-time PCR technique to performs rapid, specific, and reliable quantitation assay at the level of 10^1 copies/μL. For extraction information, please refer to the SHENTEK® Residual Host Cell DNA Sample Preparation Kit User Guide (Product No. 1104191) .

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing and gloves.

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
SV40LTA & E1A linear DNA Control	NNA019	lyophilized powder ×1 tube	-20℃
SV40LTA & E1A non-linear DNA Control	NNA020	50 μL × 1 tube	-20℃
qPCR Reaction Buffer	NNB001	850 μL × 2 tubes	-20℃, protect from light
SV40LTA & E1A Primer & Probe MIX	NNC030	300 μL × 1 tube	-20℃, protect from light
IPC MIX	NNC066	150 μL × 1 tube	-20℃, protect from light
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 3 tubes	-20℃

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Applied instruments, including but not limited to the following

➢ SHENTEK-96S Real-Time PCR System

- 7500 Real-Time PCR System
- CFX96 Real-Time PCR System

■ Required materials not included in the kit

- Nonstick, DNase-free & Low Retention Microfuge Tubes, 1.5 mL
- Nonstick, Low Retention Tips, 1000 µL, 100 µL, 10 µL
- 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps

■ Related equipment

- Bentchtop microcentrifuge
- Real-Time PCR System
- Vortex mixer
- Micropipettes, 1000 µL, 100 µL, 10 µL
- Microplate shaker

■ Workflow

Serial dilution of control DNA

⇩

Sample preparation

⇩

qPCR reaction mix preparation

⇩

qPCR amplification

⇩

Results analysis

2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Irradiate the tabletop, pipettes and tubes with UV for 30 minutes, and disinfect with 75% ethanol.

3. Thaw the kit completely at 2-8°C or melt on ice, vortex and spin briefly.

■ DNA Control serial dilutions for the standard curve

- SV40LTA & E1A DNA Control: Spin DNA Control tube for 15 seconds in a microcentrifuge. To dissolve the lyophilized powder, open the cap carefully and add 55 µL of ddH_2O to the bottom of the tube.
- Gently flick the DNA Control standard solution with finger several times, then spin for 3-5 seconds in a centrifuge. Repeat 3 times to fully dissolve the lyophilized powder in the solution. Leave the tube stand for 10 min before use.

Calculations: Plasmid copy numbers (copies/µL) = 6.02 × 10^{14} × Plasmid concentration (ng/µL) / (Number of plasmid bases × 660)

SV40LTA linear DNA Control: 4.67 × 10^9 copies/µL

E1A linear DNA Control: 4.97 × 10^9 copies/µL

SV40LTA non-linear DNA Control: 2.80 × 10^9 copies/µL

E1A non-linear DNA Control: 2.98 × 10^9 copies/µL

1. Thaw SV40LTA & E1A DNA Control and DNA Dilution Buffer (DDB) completely at 2-8℃ or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times.
2. If SV40LTA & E1A non-linear DNA Control is chosen, label seven nonstick 1.5 mL microfuge tubes: ST, ST0, ST1, ST2, ST3, ST4 and ST5. If SV40LTA & E1A linear DNA Control is chosen, label eight nonstick 1.5 mL microfuge tubes: ST, ST0, ST1, ST2, ST3, ST4, ST5 and ST6.
3. Dilute the SV40LTA & E1A DNA Control with DDB in the ST tube. Vortex to mix well and quickly spin down the mixture for 3-5 seconds in microcentrifuge, and mix thoroughly by repeating 3 times.
4. Add 90µL DDB to each tube: ST1, ST2, ST3, ST4, ST5 and ST6.
5. Perform the serial dilution according to Table 2:

Table 2. Dilution for SV40LTA & E1A DNA Control

Serial dilution tube	Dilution	Conc. (copies/μL)			
		non-linear		linear	
		SV40LTA	E1A	SV40LTA	E1A
ST	10 μL DNA Control + 90 μL DDB	2.80×10^8	2.98×10^8	4.67×10^8	4.97×10^8
ST0	10 μL ST + 90 μL DDB	2.80×10^7	2.98×10^7	4.67×10^7	4.97×10^7
ST1	10 μL ST0 + 90 μL DDB	2.80×10^6	2.98×10^6	4.67×10^6	4.97×10^6
ST2	10 μL ST1 + 90 μL DDB	2.80×10^5	2.98×10^5	4.67×10^5	4.97×10^5
ST3	10 μL ST2 + 90 μL DDB	2.80×10^4	2.98×10^4	4.67×10^4	4.97×10^4
ST4	10 μL ST3 + 90 μLDDB	2.80×10^3	2.98×10^3	4.67×10^3	4.97×10^3
ST5	10 μL ST4 + 90 μL DDB	2.80×10^2	2.98×10^2	4.67×10^2	4.97×10^2
ST6	10 μL ST5 + 90 μL DDB	/	/	4.67×10^1	4.97×10^1

- *The remaining unused DDB need to be stored at 2-8℃. If the solution is cloudy or contains precipitates, heat at 37℃ until it clear.*
- *At least five concentration of standard curve should be included. To select appropriate sample dilutions, we recommend to perform method validation before sample testing.*

■ Sample preparation

➢ Negative Control Sample (NCS) Preparation

Add 100 μL of DDB to a new 1.5 mL clean centrifuge tube, and label as NCS.

NCS and samples should be prepared in same way for DNA extraction.

■ qPCR MIX preparation

1. Determine the number of reaction wells based on your selected standard curve, with the number of test samples and control samples. Generally, triplicates are tested for each sample.

   Number of reaction wells = (5 standard points on the standard curve + 1 NTC + 1 NCS + test samples) × 3

2. Prepare qPCR MIX according to the number of reaction wells.

Table 3. qPCR MIX Preparation

Reagents	Volume for 1 reaction	Volume for 30 reactions (includes 10% overage)
qPCR Reaction Buffer	15.9 μL	524.7 μL
SV40LTA & E1A Primer & Probe MIX	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

3. Mix thoroughly and place on ice, aliquot 20 μL/well into PCR 8-strip tubes or 96-well qPCR plate.

■ qPCR Reaction MIX preparation

1. Prepare qPCR Reaction MIX according to Table 4 and 96-well plate layout as shown in Table 5.

Table 4. qPCR Reaction MIX Preparation

Tubes	Standard curve	NTC	NCS	Test sample
qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST5	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 5. Example of 96-well Plate layout

S1	S1	S1										A
S2	S2	S2										B
S3	S3	S3							ST5	ST5	ST5	C
S4	S4	S4							ST4	ST4	ST4	D
S5	S5	S5							ST3	ST3	ST3	E
									ST2	ST2	ST2	F
NTC	NTC	NTC							ST1	ST1	ST1	G
NCS	NCS	NCS										H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents the assay for a standard curve with 5 concentration gradients (ST1-ST5), 1 NTC, 1 NCS, 5 test samples (S1- S5) and 3 replicates for each sample.*

- *In specific testing, the plate layout for sample loading can be adjusted based on the sample quantity. Please refer to the example shown in Table 5.*

2. Seal the 96-well plate with sealing film. Mix it well in a microplate shaker, then spin down the reagents for 10 seconds in centrifuge and place it onto the qPCR instrument.

■ qPCR program setting

NOTE: The following instructions apply only to the ABI7500 instrument with SDS v1.4. If you use a different instrument or software, refer to the applicable instrument or software documentation.

1. Create a new document, then in the Assay drop-down list, select Standard Curve **(Absolute Quantitation)**.
2. In the Run Mode drop-down list, select **Standard 7500**, then click **Next.**
3. Click New Detector, then enter SV40LTA-DNA in the Name field, select FAM in the Reporter Dye drop-down list and select (none) in the Quencher; Click New Detector, then enter E1A-DNA in the Name field, select CY5 in the Reporter Dye drop-down list and select (none) in the Quencher; Click New Detector, then enter IPC in the Name field. Select VIC in the Reporter Dye drop-down list and select (none) in the Quencher Dye drop-down list, then click OK.
4. Select **ROX** as the passive reference dye, then Click **Next**.
5. Select the applicable set of wells for the samples, then select the corresponding detector for each well.
6. Select Finish, and then set thermal-cycling conditions:
   a. Set the thermal cycling reaction volume to 30 μL.
   b. Set the qPCR program as Table 6:

Table 6. qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Activation	95℃	10:00	1
Denaturation	95℃	00:15	40
Annealing/extension	60℃*	1:00	

* Instrument will read the fluorescence signal during this step.

7. Save the document, then click **Start** to start the real-time qPCR run.

■ Results analysis

1. Select **Set up** tab, then set tasks for each sample type by clicking on the Task Column drop-down list:
   a. NTC: target DNA detector task = **NTC**
   b. NCS, test samples: target DNA detector task = **Unknown**
2. Set up the standard curve as shown in Table 7:

Table 7. Settings for Standard curve

Tube label	Task	non-linear DNA (copies/μL)		linear DNA (copies/μL)	
		SV40LTA	E1A	SV40LTA	E1A
ST1	Standard	2.80×10^6	2.98×10^6	4.67×10^6	4.97×10^6
ST2	Standard	2.80×10^5	2.98×10^5	4.67×10^5	4.97×10^5
ST3	Standard	2.80×10^4	2.98×10^4	4.67×10^4	4.97×10^4
ST4	Standard	2.80×10^3	2.98×10^3	4.67×10^3	4.97×10^3
ST5	Standard	2.80×10^2	2.98×10^2	4.67×10^2	4.97×10^2
ST6	Standard	/	/	4.67×10^1	4.97×10^1

3. Select the **Results** tab, then select Amplification Plot.
4. In the Data drop-down list, select **Delta Rn vs Cycle.**
5. In the Analysis Settings window, enter the following settings:
   a. Select **Manual Ct**.
   b. In the Threshold field, enter 0.02.
   c. Select **Automatic Baseline**.
6. Click the button ▶ in the toolbar, then wait the plate analyzing.
7. Select the **Result** tab**> >Standard curve** tab, then verify the Slope, Intercept and R^2 values.
8. Select the Report tab, then achieve the mean quantity and standard deviation for each sample.
9. Select **File > > Export > > Results**. In the Save as type drop-down list, select

Results Export Files, then click **Save**.

10. In the Report panel of Results, the 'Mean Quantity' column shows the detection values of NTC, NCS, test sample.
11. The Ct value of IPC need to be anaylzed. In princeple, the mean Ct-IPC value of the sample should be within ±1.0 of the NCS Ct-IPC value. If the mean Ct-IPC value of the sample is significantly higher than the NCS, this indicates that the sample may be inhibitory to the assay. If you have included ERC sample in parallel, then consider sample recovery rate prior to IPC results, and IPC results can only be used as reference.
12. The SV40LTA detection value of NTC should be no more than 14.32 copies/μL, the E1A detection value of NTC should be no more than 17.79 copies/μL or set criteria by your own validation methods.The Ct value of NCS should be larger than the Ct value of the minimum standard curve concentration, and if the proven limit of quantification concentration is lower than the minimum standard curve concentration, the detected value of NCS should be less than the limit of quantification concentration.

Note: The parameter settings of the result analysis should be configured on the specific model and the software version, and in principle can also be automatically interpreted by the instrument.

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Support & Contact

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