Residual E1A DNA Quantitation Kit
User Guide

Version: A/0
For Research Use Only
Product No.: 1101109
Reagents for 100 Reactions

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

1. Product information

■ Product description

SHENTEK® Residual E1A DNA Quantitation Kit is used to quantitate residual E1A DNA from host cell, such as HEK293 and 293T cells, in different stages of biopharmaceutical products, from in-process samples to final products. This kit utilizes fluorescent quantitative PCR technique to perform rapid, specific, and reliable quantitation assay with residual E1A DNA. IPC (Internal Positive Control) is included in the kit to evaluate the performance of each PCR reaction. The kit provides E1A linear DNA Control and E1A non-linear DNA Control for customers to choose on their own needs. 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
E1A linear DNA Control	NNA012	lyophilized powder × 1 tube	-20℃
E1A non-linear DNA Control	NNA013	50 μL × 1 tube	-20℃
qPCR Reaction Buffer	NNB001	850 μL × 2 tubes	-20℃, protect from light
E1A Primer&Probe MIX	NNC033	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 and 10 µL

➢96-well qPCR plates with sealing film or PCR 8-strip tubes with caps

■ Related equipment

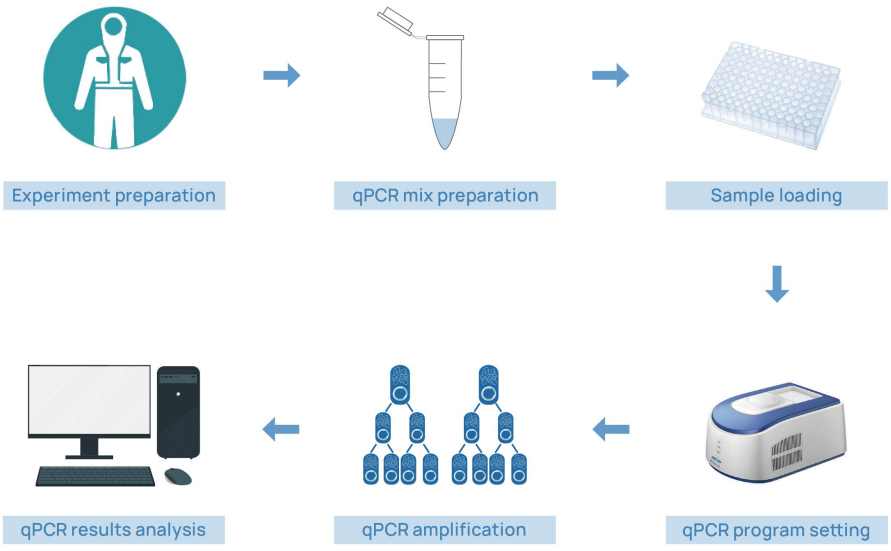
➢Real-Time PCR System

➢Vortex mixer

➢Desktop microcentrifuge

➢Micropipettes: 1000 µL, 100 µL and 10 µL

■ Workflow



2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Irradiate the tabletop, micropipettes 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

Please check the concentration labeled on the tubes containing E1A linear DNA Control and E1A non-linear DNA Control prior to dilution.

Thaw DNA Dilution Buffer (DDB) completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times.

E1A linear DNA Control:

1. Rapidly centrifuge E1A linear DNA Control for 15 seconds, add precisely 55 μL ddH_2O to the bottom of the tube.

2. In order to ensure that the lyophilized powder is fully dissolved, flick few times to mix well and centrifuge breifly for 10 seconds, repeat for 3 times, and let it stand for 10 min before use.

3. Label eight nonstick 1.5 mL microfuge tubes: ST, ST0, ST1, ST2, ST3, ST4, ST5 and ST6.

4. Dilute E1A linear DNA Control to 4.97×10^8 copies/μL with DDB in the ST tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.

5. Then dilute ST sample 10-fold with DDB in the ST0 tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times to ensure fully mixed.

6. Add 90 μL DDB to each tube of ST1, ST2, ST3, ST4, ST5 and ST6.

7. Perform the serial dilutions according to Table 2:

E1A non-linear DNA Control:

1. Thaw E1A non-linear DNA Control completely at 2-8°C 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. Label six nonstick 1.5 mL microfuge tubes: ST0, ST1, ST2, ST3, ST4 and ST5.

3. Dilute E1A non-linear DNA Control to 2.98×10^7 copies/μL with DDB in the ST0 tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.

4. Add 90 μL DDB to each tube of ST1, ST2, ST3, ST4 and ST5.

5. Perform the serial dilutions according to Table 2:

Table 2. Dilution for E1A linear DNA Control and E1A non-linear DNA Control

Serial dilution tube	**Dilution**	**Conc. (copies/μL)**	
		non-linear DNA	**linear DNA**
ST1	10 μL ST0 + 90 μL DDB	2.98×10^6	4.97×10^6
ST2	10 μL ST1 + 90 μL DDB	2.98×10^5	4.97×10^5
ST3	10 μL ST2 + 90 μL DDB	2.98×10^4	4.97×10^4
ST4	10 μL ST3 + 90 μL DDB	2.98×10^3	4.97×10^3
ST5	10 μL ST4 + 90 μL DDB	2.98×10^2	4.97×10^2
ST6	10 μL ST5 + 90 μL DDB	/	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

- Test Sample Preparation

  Take 100 μL of each test sample and add it to a new 1.5 mL microfuge tube.

- Negative Control Sample (NCS) Preparation

  Add 100 μL of DDB to a new 1.5 mL microfuge 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

   If E1A linear DNA Control is used, the standard curve should include 6 concentration gradients.

2. Prepare qPCR MIX according to the number of reaction wells in Table 3.

Table 3. qPCR MIX Preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
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 96-well qPCR plate or PCR 8-strip tubes.

■ qPCR Reaction MIX preparation

1. Prepare qPCR Reaction MIX according to Table 4, and 96-well plate layout is 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

*If E1A linear DNA Control is used, the standard curve should include 6 concentration gradients.

Table 5. Example of 96-well Plate layout

S1	S1	S1										A
S2	S2	S2										B
S3	S3	S3							ST1	ST1	ST1	C
S4	S4	S4							ST2	ST2	ST2	D
S5	S5	S5							ST3	ST3	ST3	E
									ST4	ST4	ST4	F
									ST5	ST5	ST5	G
NCS	NCS	NCS	NTC	NTC	NTC							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 to ST5), 1 NTC, 1 NCS, and 5 test samples (S1 to S5), with 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 microplate shaker, then spin down the reagents for 10 seconds in centrifuge and place it on 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:**
   a. Enter E1A-DNA in the Name field.
   b. Select **CY5** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
   c. Select a color for the detector, then click **Create Another**.
4. Click **New Detector:**
   a. Enter IPC in the Name field.
   b. Select **VIC** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
   c. Select a color for the detector, then click **OK**.
   d. Select the detectors, then click **Add** to add the detectors to the document.
5. Select **ROX** as the passive reference dye, then Click **Next**.
6. Select the applicable set of wells for the samples, then select E1A-DNA detector and IPC detector for each well.
7. Select Finish, and then set thermal-cycling conditions:
   a. Set the thermal cycling reaction volume to 30 μL.
   b. Set the temperature and time as following (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.

8. 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 and test samples wells: 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	Quantity (copies/μL)	
		non-linear DNA	**linear DNA**
ST1	Standard	2.98×10^6	4.97×10^6
ST2	Standard	2.98×10^5	4.97×10^5
ST3	Standard	2.98×10^4	4.97×10^4
ST4	Standard	2.98×10^3	4.97×10^3
ST5	Standard	2.98×10^2	4.97×10^2
ST6	Standard	/	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, E1A-DNA and IPC 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 and test samples, in copies/μL.
11. The Ct value of NTC should be no less than 35.00 cycles or undetermined.If the proven limit of quantification (LOQ) concentration is lower than the lowest concentration of the standard curve, the Ct value of NCS should be larger than the Ct value of LOQ.
12. The Ct-IPC value of the sample and the Ct-IPC value of the NCS should be within the range of ±1.0 Ct value. If the Ct-IPC value of the sample is significantly higher than the Ct-IPC value of the NCS, it indicates that the sample may be inhibited. If the samples that include E1A DNA spike concentration are tested at the same time, please consider the recovery rate as priority, and take the IPC results as reference.

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

Effective date: 17 May 2024