SHENTEK

Residual Plasmid DNA Quantitation Kit (3G) User Guide

Version: A/1
For Research Use Only
Product No.: 1101111-1
Reagents for 100 Reactions

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

1. Product information

■ Product description

SHENTEK® Residual Plasmid DNA Quantitation Kit (3G) is used to quantitate plasmid DNA residues in gene therapy products, such as the plasmid DNA of lentiviral vector preparation in CAR-T cell therapy. This kit uses duplex real-time PCR technology to detect trace amounts of residual plasmid DNA by means of consensual plasmid sequences, such as replicon of ColE1/pMB1/pBR322/pUC. The target gene (FAM) performs rapid, specific, and reliable quantitative determination of the 10^2 copies/μL level of residual Plasmid DNA. IPC—Internal Positive Control (VIC) is included in the Plasmid Primer&Probe MIX to evaluate the performance of each PCR reaction. Customers can send DNA sequence of empty plasmid backbones to our technicians for confirmation in advance. 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
Plasmid linear DNA Control	NNA016	lyophilized powder ×1 tube	-20℃
Plasmid Primer&Probe MIX (Incl IPC)	NNC120	500 μL × 1 tube	-20℃, protect from light
qPCR Master MIX	NNB023	850 μL × 2 tubes	-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
- Lightcycler 480 Real-Time PCR System

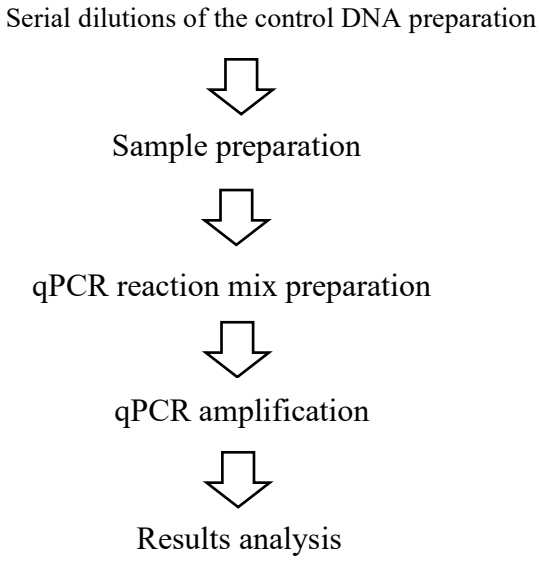
■ Required materials not included in the kit

- 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 or PCR 8-strip tubes

■ Related equipment

- Real-Time PCR System
- Vortex mixer
- Microplate shaker
- Pipettes, 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, 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

- Spin Plasmid linear DNA Control for 15 seconds in a centrifuge and then add 55 μL of ddH_2O accurately to the bottom of the tube to dissolve the lyophilized powder.
- Gently flick the Plasmid linear 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.

  Please check the concentration on the label of the tube containing the Plasimd linear DNA Control prior to dilution.

  1. Thaw Plasmid linear DNA Control and DNA Dilution Buffer 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. Label eight nonstick 1.5 mL microfuge tubes: A, B, C, ST1, ST2, ST3, ST4, ST5.
  3. Dilute the Plasmid linear DNA Control to 4.97×10^8 copies/μL with DDB in the A tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times to mix it thoroughly.
  4. Add 90μL DDB to each tube: B, C, ST1, ST2, ST3, ST4, ST5.
  5. Perform the serial dilution:

Table 2. Dilution for plasmid linear DNA Control

Serial Dilution Tube	Dilution	Conc.(copies/μL)
A	Dilute the DNA control with DDB	4.97×10^{8}
B	10 μL A + 90 μL DDB	4.97×10^{7}
C	10 μL B + 90 μL DDB	4.97×10^{6}
ST1	10 μL C + 90 μL DDB	4.97×10^{5}
ST2	10 μL ST1 + 90 μL DDB	4.97×10^{4}
ST3	10 μL ST2 + 90 μL DDB	4.97×10^{3}
ST4	10 μL ST3 + 90 μL DDB	4.97×10^{2}
ST5	10 μL ST4 + 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 clears.*
- *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 the test sample and add it to a new 1.5 mL centrifuge tube.

➢ Extraction Reference Control (ERC) samples Preparation

According to the Plasmid DNA spike concentration in ERC samples (Take the samples containing 4.97×10^{5} copies of Plasmid DNA for example), the specific preparation procedure is as follows:

(1) Take 100 μL of the test sample and add it to a new 1.5 mL centrifuge tube.

(2) Add another 10 μL of ST2, mix thoroughly and lable it as the ERC sample.

➢ Negative Control Sample (NCS) Preparation

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

■ qPCR MIX preparation

1. Determine the number of reaction wells based on the 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

Reagent	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Master MIX	15 μL	495 μL
Plasmid Primer&Probe MIX (Incl IPC)	5 μL	165 μL
Total volume	20 μL	660 μL

3. After thoroughly mixing qPCR MIX, follow 20 μL each tube is divided 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

Standard curve	20 μL qPCR MIX +10 μL ST1/ST2/ST3/ST4/ ST5
NTC	20 μL qPCR MIX + 10 μL DDB
NCS	20 μL qPCR MIX + 10 μL purified NCS
Test sample	20 μL qPCR MIX + 10 μL purified test sample
Test sample ERC	20 μL qPCR MIX + 10 μL purified ERC sample

Table 5. Example of 96-well Plate layout

NTC		S1	S1	S1	S1 ERC	S1 ERC	S1 ERC		ST5	ST5	ST5	A
NTC		S2	S2	S2	S2 ERC	S2 ERC	S2 ERC		ST4	ST4	ST4	B
NTC		S3	S3	S3	S3 ERC	S3 ERC	S3 ERC		ST3	ST3	ST3	C
		S4	S4	S4	S4 ERC	S4 ERC	S4 ERC		ST2	ST2	ST2	D
NCS		S5	S5	S5	S5 ERC	S5 ERC	S5 ERC		ST1	ST1	ST1	E
NCS												F
NCS												G
												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, 5 test samples (S1 to S5), 5 ERC samples (S1 ERC to S5 ERC), 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 microplate shaker, then spin down the reagents for 10 seconds in centrifuge and place it in 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 Plasmid-DNA in the Name field.
   b. Select **FAM** 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 Plasmid-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 the time as following:

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, test samples, and ERC wells: target DNA detector task = **Unknown**

2. Set up the standard curve as shown in the following table:

Table 7. Settings for standard curve

Tube label	Task	Quantity (copies/μL)
ST1	Standard	4.97×10^5
ST2	Standard	4.97×10^4
ST3	Standard	4.97×10^3
ST4	Standard	4.97×10^2
ST5	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, Plasmid-DNA enter 0.05 and IPC enter 0.1.
   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 can read the detection values of NTC, NCS, test sample, and ERC sample, in copies/μL.
11. The recovery rate of ERC samples should be calculated based on the test results of the test samples and the ERC samples. The recovery rates should be between 50% and 150%.
12. The Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve, and it shows normal amplification curve in the VIC signal channel.
13. The Ct value of NTC should be 2 larger than the Ct value of ST5, or set specific standards based on the laboratory's own validation results, and it shows

normal amplification curve in the VIC signal channel.

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