SHENTEK

ResHCD™ Plasmid DNA Quantitation Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1101120
Reagents for 100 Reactions

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

1. Product information

■ Product description

The ResHCD™ Plasmid DNA Quantitation Kit is used to quantitate residual plasmid DNA in gene therapy products, such as the lentiviral encoding plasmid DNA in CAR-T therapy. This kit utilizes real-time PCR technique to detect the amount of residual plasmid DNA by targeting consensual sequences of common plasmids, such as ColE1/pMB1/pBR322/pUC derived replicons. For extraction information, please refer to the SHENTEK® Residual Host Cell DNA Sample Preparation Kit (Fast) User Guide (Product No. 1104201).

The kit is for Research Use Only and is not for use in diagnostic procedures or for therapeutic purposes in humans or animals.

■ Kit components and storage

WARNING: Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing and gloves.

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
qPCR Reaction MIX-1	NNB028	850 µL × 2 tubes	-20℃, protect from light
Plasmid Primer&Probe MIX	NNC031	300 µL × 1 tube	
IPC MIX-6	NNC145	200 µL × 1 tube	
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 3 tubes	-20℃
Plasmid linear DNA Control	NNA016	1 tube	

Note: According to USP <659>, items with recommended storage temperatures not exceeding –20°C shall be stored within ± 10°C (–30°C to –10°C). Short-term storage below –30°C is acceptable when supported by stability data.

The kit components can be stored under the 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-96H Real-Time PCR System

➢ABI 7500 Real-Time PCR System

➢LightCycler 480 II Real-Time PCR System

■ Required materials not included in the kit

➢Low retention, RNase/DNase-free, sterile microcentrifuge tubes

➢96-well qPCR plates with sealing film, or 8-tube PCR strips with caps

➢Low retention filter tips: 1000 µL, 100 µL and 10 µL

■ Related equipments

➢Real-Time PCR System

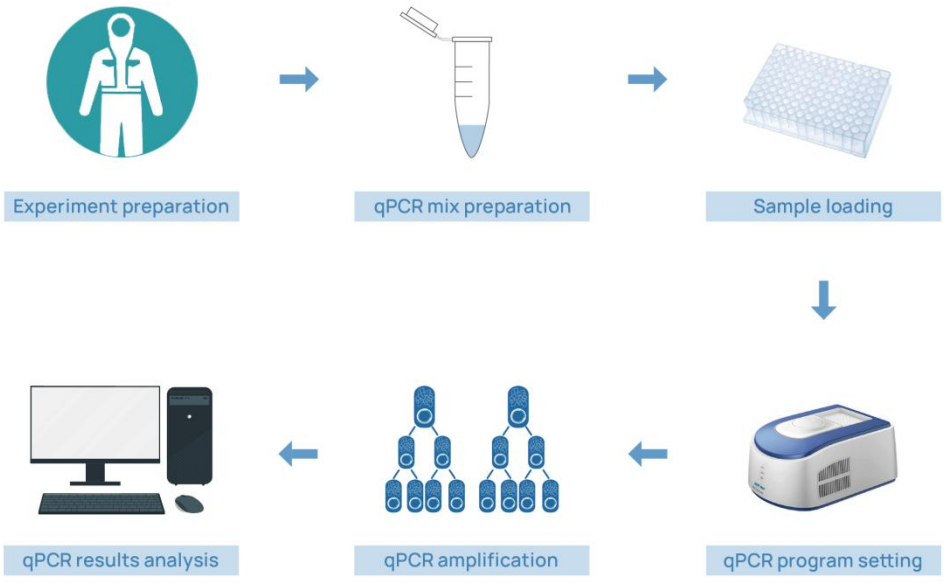
➢Vortex mixer

➢Benchtop microcentrifuge

➢Pipettes: 1000 µL, 100 µL and 10 µL

➢Microplate and microtube shaker

■ 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 centrifuge briefly.

■ DNA Control serial dilutions for the standard curve

- Spin Plasmid linear DNA Control for 15 seconds in a microcentrifuge 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 labeled on the tube containing the Plasmid 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 1.5 mL microcentrifuge 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.

Calculate the volume of DDB to prepare the A tube:

$$\frac{\text{DNA Control conc. (A)} \times \text{Volume of DNA Control (B)}}{4.97\times10^8 \text{ copies/µL}} - \text{Volume of DNA Control (B)}$$

For example:

The concentration on the label of the DNA Control is 5.21×10^9 copies/µL (A), pipette 10 µL (B) of the DNA Control to the A tube. Add the below volume to reach 4.97×10^8 copies/µL.

$$\frac{5.21\times10^{9}\ \text{copies}/\mu\text{L}\times 10\ \mu\text{L}}{4.97\times10^{8}\ \text{copies}/\mu\text{L}} - 10\ \mu\text{L}=94.8\ \mu\text{L}$$

4. Add 90μL DDB to each tube: B, C, ST1, ST2, ST3, ST4, ST5.
5. Perform the serial dilution according to Table 2.

Table 2. Dilution for Plasmid 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}

Note: 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.

Note: At least five concentration values should be included when creating a standard curve. To select appropriate sample dilutions, we recommend performing method validation before sample testing.

■ Sample preparation

➢ Preparation for Extraction Recovery Control (ERC) samples

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

1. Add 100 μL of the test sample to a new 1.5 mL microcentrifuge tube.
2. Add 10 μL of ST2 solution and mix thoroughly, label it as the “ERC sample”.

➢ Preparation for Negative Control Samples (NCS)

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

The ERC sample and NCS should be processed in the same procedures as test sample preparation before testing.

■ qPCR Reaction 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 = (standard curve of 5 concentration gradients + 1 NTC + 1 NCS + test samples) × 3.

2. Prepare qPCR Reaction MIX according to Table 3 and Table 4.

3. Refer to 96-well plate layouts shown in Table 5.

Table 3. qPCR MIX Preparation

Reagents	**Volume/reaction**	**Volume for 30 reactions (includes 10% overage)**
qPCR Reaction MIX-1	15 μL	495 μL
Plasmid Primer&Probe MIX	3 μL	99 μL
IPC MIX-6	2 μL	66 μL
Total volume	20 μL	660 μL

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
ERC sample	20 μL qPCR MIX + 10 μL purified ERC sample

Table 5. Example of 96-well plate layouts

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	

Note: 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), and 5 ERC samples (S1 ERC to S5 ERC), and 3 replicates for each sample.

Note: In specific testing, the plate layouts for sample loading can be adjusted based on the sample quantity.

4. Seal the 96-well plate with sealing film. Mix contents of the wells in microplate shaker, then spin down the plate for 10 seconds in microcentrifuge and place it in the qPCR instrument.

■ qPCR program setting

Note: The following instructions apply only to the ABI 7500 Real-Time PCR System with SDS v1.5.1. 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 and IPC detector for each well.

7. Select **Finish**, and then set thermal-cycling conditions:

a. Choose the thermal cycling reaction volume to 30 μL.

b. Set up the program 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	Conc. (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, enter value 0.05 of FAM; value 0.1 of VIC.
   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. The recovery of ERC samples is calculated based on the value of the test samples and the ERC samples. The recovery should be between 50% and 150%.
11. The Ct value of NCS should be higher than the mean Ct value of the lowest concentration in the standard curve. If the validated quantitation limit (QL) concentration is lower than the lowest concentration in the standard curve, the value of the NCS should be below the QL.
12. The Ct value of NTC should be not lower than the Ct value of ST5, or set specific standards based on the laboratory's own validation results.

Note: The parameter settings listed above are generally recommended for result analysis and could be adjusted depending on the specific instrument model and software version. In most cases, the instrument performs the interpretation automatically.

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Support & Contact

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