SHENTEK

ResHCD™ Human DNA Quantitation Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1101119
Reagents for 100 Reactions

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

1. Product information

■ Product description

The ResHCD™ Human DNA Quantitation Kit is used for quantitation of residual Human host cell DNA in a variety of biopharmaceutical products. By combining quantitative PCR, the kit ensures a rapid and specific quantitation of residual DNA at the femtogram level. 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-2	NNB029	850 µL × 2 tubes	-20℃, protect from light
Human Primer&Probe MIX	NNC007	300 µL × 1 tube	
IPC MIX-4	NNC143	200 µL × 1 tube	
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 3 tubes	-20℃
Human DNA Control	NNA003	50 µL × 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

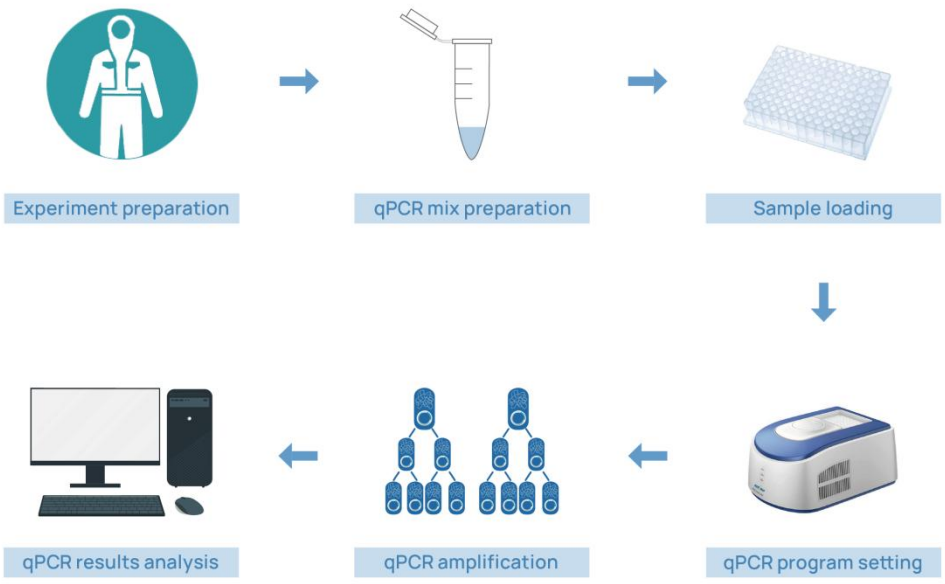
■ Required materials not included in the kit

- 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 spin briefly.

■ DNA Control serial dilutions for the standard curve

Please check the concentration labeled on the tube containing the Human DNA Control prior to dilution.

Prepare a series of Human DNA Control solution with DNA Dilution Buffer (DDB) as follows:

1. Thaw Human DNA Control and DNA Dilution Buffer completely at 2-8°C or melt on ice. Vortex to mix well and centrifuge briefly, repeat 3 times.
2. Label six 1.5 mL microcentrifuge tubes as ST0, ST1, ST2, ST3, ST4 and ST5 respectively.
3. Dilute the Human DNA Control to 3000 pg/μL with DDB in the ST0 tube.

   Calculate the volume of DDB to prepare the ST0:

$$\frac{\text{DNA Control conc. (A)} \times 1000\ \text{pg/ng} \times \text{Volume of DNA Control (B)}}{3000\ \text{pg/μL}} - \text{Volume of DNA Control (B)}$$

   For example:

   The concentration on the label of the DNA Control is 30.9 ng/μL (A), pipette 10 μL (B) of the DNA Control to the ST0 tube. Add DDB in the volume shown in the calculation below to reach a final concentration of 3000 pg/μL.

$$\frac{30.9\ \text{ng/μL} \times 1000\ \text{pg/ng} \times 10\ \text{μL}}{3000\ \text{pg/μL}} - 10\ \text{μL} = 93\ \text{μL}$$

4. Vortex to mix well and centrifuge briefly the ST0 tube for 3-5 seconds in microcentrifuge,, and repeat 3 times to mix thoroughly.
5. Add 90 μL DDB to each tube of ST1, ST2, ST3, ST4 and ST5.
6. Perform the serial dilutions according to Table 2:

Table 2. Dilution for Human DNA Control

Serial dilution tube	Dilution	Conc. (pg/μL)
ST0	Dilute the DNA control with DDB	3000
ST1	10μL ST0 + 90μL DDB	300
ST2	10μL ST1 + 90μL DDB	30
ST3	10μL ST2 + 90μL DDB	3
ST4	10μL ST3 + 90μL DDB	0.3
ST5	10μL ST4 + 90μL DDB	0.03

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 Human DNA spike concentration in ERC samples (Take the samples containing 30 pg of Human DNA as an example), the specific preparation procedure is as follows:

  1. Aliquot 100 μL of the test sample to a new 1.5 mL microcentrifuge tube.
  2. Add 10 μL of ST3 solution and mix thoroughly, label it as “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-2	15 μL	495 μL
Human Primer&Probe MIX	3 μL	99 μL
IPC MIX-4	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 sample (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 ABI7500 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 **Human-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 Human-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. (pg/μL)
ST1	Standard	300
ST2	Standard	30
ST3	Standard	3
ST4	Standard	0.3
ST5	Standard	0.03

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 detection value of NTC should be no more than 6.00 fg/μL, or set specific criteria by your own method validation, meanwhile shows normal

amplification curve in the VIC signal channel.

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.

Effective date: 20 Apr. 2026

Support & Contact

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