SHENTEK

Residual Human DNA Quantitation Kit
User Guide

Version: A/1
For Research Use Only
Product No.: SK030207H100
Reagents for 100 Reactions

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

1. Product information

■ Product description

SHENTEK® Residual Human DNA Quantitation Kit is used to quantitate residual Human host cell DNA of biopharmaceutical products in different stages, from in-process samples to final products. This kit uses duplex real-time PCR technique to performs rapid, specific, and reliable quantitation assay at femtogram (fg) level. 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
Human DNA Control	NNA003	50 μL × 1 tube	-20℃
qPCR Reaction Buffer	NNB001	850 μL × 2 tubes	-20℃, protect from light
Human Primer&Probe MIX	NNC007	300 μL × 1 tube	
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

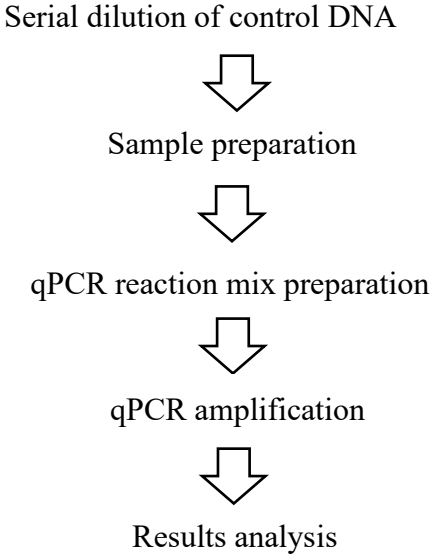
■ Required materials not included in the kit

- 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

- Benchtop microcentrifuge
- Real-Time PCR system
- Vortex mixer
- Microplate shaker
- 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, 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.

■ Human DNA Control serial dilutions for the standard curve

Please check the concentration on the label of Human DNA Control tube prior to dilution.

1. Thaw Human DNA Control and 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.
2. Label six nonstick 1.5 mL microfuge tubes: ST0, ST1, ST2, ST3, ST4 and ST5.
3. Dilute the DNA Control to 3000 pg/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 it 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 Human DNA Control

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

- *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 the test sample(s) and add to a new 1.5 mL centrifuge tube.

- Extraction Reference Control (ERC) sample preparation

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

  (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 ST3, mix thoroughly and label as the ERC sample.

- Negative Control Sample (NCS) Preparation

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

■ 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	17 μL	561 μL
Human Primer&Probe MIX	3 μL	99 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

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 well in a microplate shaker, then spin down the reagents for 10 seconds 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:**
   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. Select **ROX** as the passive reference dye, then Click **Next**.
5. Select the applicable set of wells for the samples, then select Human-DNA detector.
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 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.

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, 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 (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, Human-DNA 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, and sample ERC, in pg/μL.
11. Thehe recovery rate of ERC samples is calculated based on the value 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 shows normal amplification curve in the VIC signal channel.
13. The detection value of NTC should be no more than 6.00 fg/μL, or set specific criteria by your own validation methods.

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

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Support & Contact

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