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Residual Human DNA Quantitation Kit User Guide

Version: A/2

Product No.: SK030207H100

Reagents for 100 Reactions

For Research Use Only. Not for diagnostic or therapeutic use.

SHENTEK® Residual Human DNA Quantitation Kit

(PCR-Fluorescence Probe Method)

Product No.: SK030207H100

Reagents for 100 Reactions

⚠	**WARNING!** Read this user guide thoroughly before starting the experiment. Pay close attention to the precautions and frequently asked questions.
⚠	**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing and gloves.

Product information

Product description

The **SHENTEK® Residual Human DNA Quantitation Kit** serves to the quantitative detection of the residual host cell DNA in various samples derived from various biologic and pharmaceutical processes such as in-process samples, bulk harvest, and final product.
This kit utilizes a fluorescent probe-based qPCR principle to quantitatively detect specific genomic regions of host cell, providing rapid detection with high specificity and reliable performance, limitation of detection reaches the femtogram (fg) level for human residual DNA in various sample types.

Compatible Kit:
- SHENTEK® Residual Host Cell DNA Sample Preparation Kit (Fast) (Product No.: 1104201)
  Enable efficient recovery of residual DNA from a broad range of products across different manufacturing stages.

Kit components and storage

Reagent	Part No.	Volume	Storage
Human DNA Control	NNA003	1 × 50 µL	-20°C*
DNA Dilution Buffer (DDB)	NND001	3 × 1.5 mL	-20°C
Human Primer&Probe MIX	NNC007	1 × 300 µL	-20°C, protect from light
qPCR Reaction Buffer	NNB001	2 × 850 µL	-20°C, protect from light

*-30°C to -10°C is acceptable per USP <659>. Storage below -30°C is allowed short-term with stability support. Up to 24 months storage under proper conditions. Check the label for expiry date.

Materials required

Compatible Instruments (including but not limited to)	SHENTEK-96H Real-time PCR System (Product No.: 161086002)
	ABI 7500 Real-Time PCR System
	CFX96 Real-Time PCR System
Equipments	Benchtop microcentrifuge
	Vortex mixer
	Pipettes
Consumables	Low retention, DNase-free, Sterile microcentrifuge tubes, 1.5 mL
	8-tube PCR strips or 96-well PCR plates
	Low retention, Sterile filter tips: 1000 µL, 100 µL and 10 µL

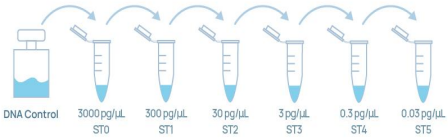
Quick Process Flow Char

01 Dilution of DNA Control and preparation of standard curves

- DNA Control
- DNA Diluent
- Primer&Probe MIX
- qPCR Reaction Buffer

Vortex

Centrifuge briefly for 3~5 seconds



Preparation of quantitative participation sample recovery quality control ERC.

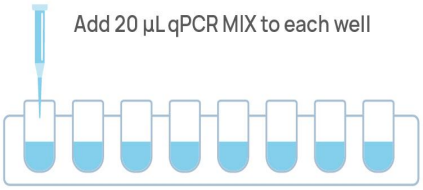
02 Preparation of qPCR premix

qPCR MIX preparation (example for a single well)

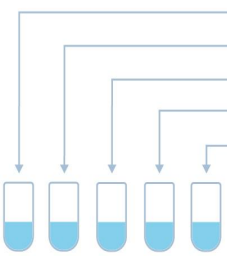
Add 17 μL qPCR Reaction Buffer

Add 3 μL Primer&Probe MIX

Add 20 μL qPCR MIX to each well



03 Sample addition

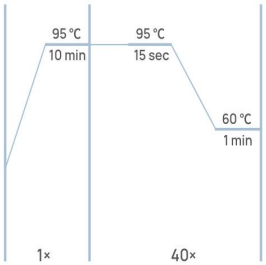


Tightly cover the tube cap or seal with a sealing film

Centrifuge for 10 seconds

Run samples in triplicates

04 qPCR Program Setup and Operation



Analysis procedure

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.
4. If the DNA Dilution Buffer (DDB) solution is cloudy or contains precipitates, heat at 37°C until it clears.

DNA Control serial dilutions for the standard curve

- 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.

1. Thaw DNA Control and DDB completely. Vortex and quickly spin down, repeat 3 times.
2. Prepare and label 1.5 mL microcentrifuge tubes as ST0, ST1, ST2, ST3, ST4 and ST5, respectively.
3. Dilute DNA Control with DNA Dilution Buffer in the tube ST0 to 3,000 pg/µL. Calculate the volume of DDB to prepare the ST0:

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

Example Calculation:

For 10 µL DNA Control (30.9 ng/µL), 93 µL of DDB will be added.

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

4. Add 90 µL of DDB to tubes ST1, ST2, ST3, ST4 and ST5, respectively. After each transfer, vortex thoroughly, then centrifuge briefly.
5. Perform serial dilution according to the table below.

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

ERC (Extraction Recovery Control)

Aliquot 100 µL of the test sample to a new 1.5 mL microcentrifuge tube and mix thoroughly with 10 µL of ST3 solution. Label the tube with “ERC”. The ERC concentration may be adjusted according to the actual testing requirement.

NCS (Negative Control Sample)

Add 100 µL of DDB to a new 1.5 mL microcentrifuge tube. Label the tube with “NCS”.

Note: Both ERC and NCS should share the same preparation procedures with the test samples.

qPCR Reaction MIX preparation

1. Based on the number of standard curve points, samples, and control samples, calculate the total number of reaction wells required for the assay. Triplicate recommended for each sample.

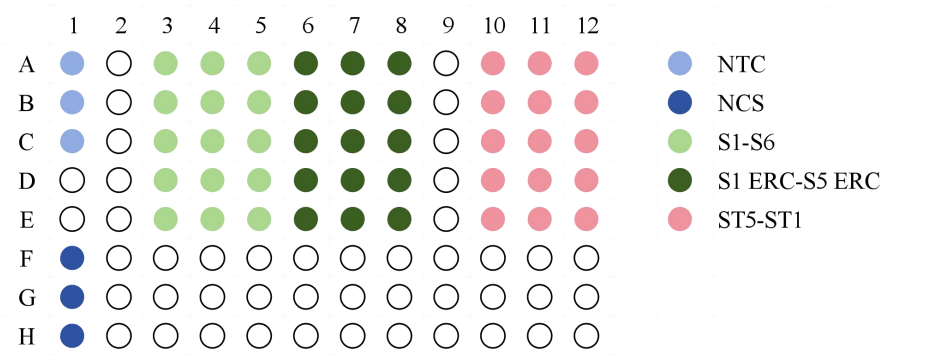
$$\text{Number of reaction wells} = (5\ \text{standard curve points} + 1\ \text{NTC} + 1\ \text{NCS} + \text{test samples}) \times 3$$

2. Thaw all components completely and thoroughly mix the reagents and briefly centrifuge. Prepare the qPCR MIX according to the reagents and volumes shown in the table below. Ensure thorough mixing and briefly centrifuge after preparation.

Component	Sample volume (1 well)	Sample volume (30 wells) *
qPCR Reaction Buffer	17 μL	561 μL
Human Primer&Probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL

*Includes 10% loss redundancy for 30 wells.

3. Dispense the prepared qPCR MIX into the 96-well plate according to the plate layout below. Add No Template Control (NTC, use DDB as the NTC in this kit), NCS, Samples, ERC, and Standards into the corresponding wells. The number of samples and plate layout can be adjusted depending on the actual testing requirement.



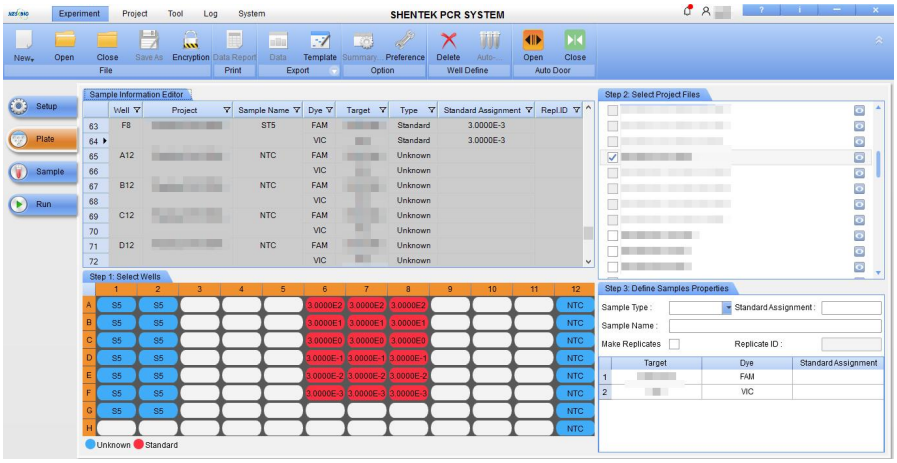
a) Add 20 μL of qPCR MIX (prepared on step 2) to each colored reaction well.

b) Sequentially add 10 μL of the following to the corresponding wells: NTC, NCS, Samples (S1-S5), ERC (S1 ERC-S5 ERC), and serial diluted Standard (ST5-ST1).

4. Close the tube lids or seal the plate with sealing film carefully. Gently vortex mix and briefly centrifuge for 10 seconds.

qPCR program setting

- The following experiment program setup procedures applies to SHENTEK-96H Real-time PCR System, software Version 9.0 example.



1. Click on “Experiment” and select “Setup”
2. Select Type “Qualitative/Absolute Quantitative” in the Experiment Information.
3. Enter the "Plate" page.
4. Select Step 1: Select wells.
5. Select Step 2: Select Project Files – “Human Residual DNA ” from list.
6. Select Step 3: Define Samples Properties, enter sample information.
7. Click "Start" to run the program on the "Run" page.

- For other quantitative PCR systems, the program setup is as follows (Refer to the applicable instrument or software documentation.):

1. Create a new blank program and select the absolute quantification detection template.
2. Create a new detection probe, name it as “Human-DNA”, define the reporting fluorophore as FAM, the quenching fluorophore as none, and the reference fluorophore as ROX (optional).

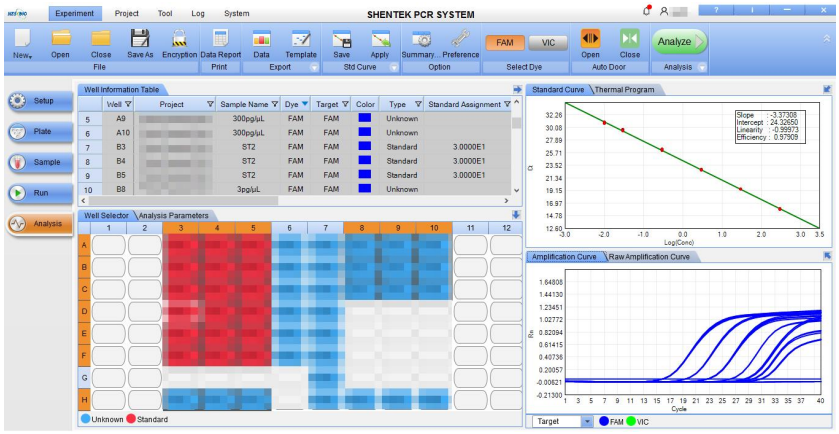
3. Set up the reaction program:

Temperature	Duration (mm: sec)	Cycle number
95°C	10: 00	1
95°C	00: 15	40
60°C*	01: 00	

*The Instrument will read the fluorescence signal during this step.

Results analysis

◆For SHENTEK-96H Real-time Fluorescent PCR System, software Version 9.0:



1. Enter the "Analysis" page, click the “Analyze” icon to check the slope, intercept, Linearity, and amplification efficiency of the standard curve.
2. In the "Well Information Table", the values for each reaction well can be displayed in units of "pg/μL".

◆For ABI 7500 Real-Time PCR System, software Version 1.5.1:

1. Enter the "Results" section and check the Amplification Plot panel. Set the Threshold to "0.02" and the Baseline to "Auto". Click "Analyze" to preliminarily check the amplification curve for normality.
2. In the "Results" section, under the Plate panel, set the Task field for the standard curve wells to "Standard". Name the corresponding wells from ST1 to ST5, and assign values based on serial dilutions table.
3. In the "Results" section, under the Plate panel, set the Task field for the NTC, NCS, Sample, and ERC to "Unknown". Name the corresponding Sample Names as NTC, NCS, S, and ERC. Then click "Analyze."
4. In the "Results" section under the Standard Curve panel, the slope, intercept, and R^2 values for the standard curve can be viewed.
5. In Report panel, the Mean Quantity field displays the detection values for NTC, NCS, ERC, and test samples, with units in pg/μL.

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.

Interpretation of results

1. Adding standard recovery calculation based on the detection results of the test samples and the ERC Sample, the recovery must fall within the range of 50% to 150%.
2. The average Ct value of the NCS should be higher than the average Ct value of the lowest concentration in the standard curve. If the verified quantitation limit (QL) concentration is lower than the lowest concentration of the standard curve, the NCS detection value should be lower than the quantitation limit (QL) concentration.
3. The NTC detection result should not exceed 6.00 fg/μL. If the laboratory has validated other acceptable thresholds, they must be clearly specified in the internal SOP, and the validation data must be retained.

Revision	Date	Description
A/0	29 Mar. 2024	Basic Version of User Guide.
A/1	08 Jul. 2024	Update Contacts & Trademark Details.
A/2	29 May 2026	Include the RUO statement and change storage instructions.

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■ Support & Contact

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou 313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189