SHENTEK

# Residual *E. coli* DNA Quantitation Kit

# User Guide

Version: A/2
For Research Use Only
Product No.: SK030202E100
Reagents for 100 Reactions

**Huzhou Shenke Biotechnology Co., Ltd**

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

# 1. Product information

## ■ Product description

SHENTEK® Residual *E.coli* DNA Quantitation Kit is used for quantitation of residual *E. coli* host cell DNA in a variety of biopharmaceutical products. This kit allows the user to perform quantitative PCR testing to ensure a rapid and specific quantitation of residual DNA at the femtogram level. The assay is accurate and reliable across a broad range of sample types, from in-process samples to final products.

The kit comes with *E. coli* DNA Control, which is traceable to a primary DNA reference standard. For extraction information, please refer to the SHENTEK® Residual Host Cell DNA Sample Preparation Kit User Guide (Product No. SK030203D100).

The product described herein is for research use only and is not intended for diagnostic or therapeutic use.

## ■ Kit components and storage

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

Table 1. Kit components and storage

| Reagent | Part No. | Quantity | Storage |
|---|---|---|---|
| *E. coli* DNA Control | NNA002 | 50 µL × 1 tube | -20℃ |
| *E. coli* qPCR MIX | NNC002 | 1 mL × 2 tubes | -20℃, protect from light |
| DNA Dilution Buffer (DDB) | NND001 | 1.5 mL × 3 tubes | -20℃ |

*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-96S Real-Time PCR System
- ABI 7500 Real-Time PCR System
- CFX96 Real-Time PCR System

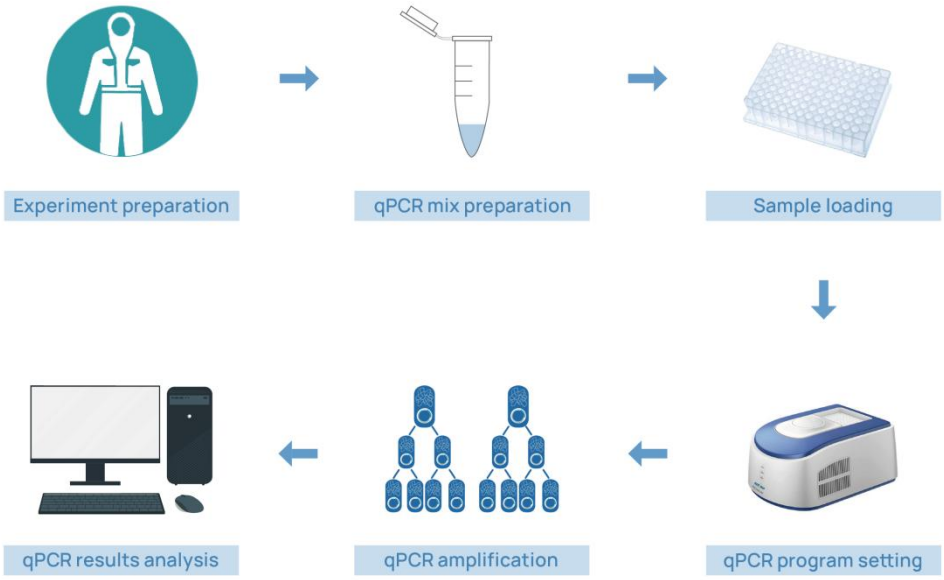
## ■ Required materials not included in the kit

- Low retention, DNase-free, Sterile microcentrifuge tubes, 1.5 mL
- 96-well qPCR plates with sealing film or 8-strip PCR tubes with caps
- Low retention, Sterile 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 on the label of the tube containing the *E. coli* DNA Control prior to dilution.

1. Thaw *E. coli* DNA Control and DNA Dilution Buffer completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down, and repeat 3 times.
2. Label six nonstick 1.5 mL microcentrifuge tubes as ST0, ST1, ST2, ST3, ST4 and ST5, respectively.
3. Transfer a certain amount of DNA Dilution Buffer and *E. coli* DNA Control to ST0 tube to achieve a 3000 pg/μL control solution. Vortex to mix well and

quickly spin down, and repeat 3 times.

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. Add 90 µL DDB to all tubes of ST1, ST2, ST3, ST4 and ST5.

5. Perform the serial dilutions according to Table 2:

Table 2. Dilution for *E. coli* 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 of Extraction Recovery Control (ERC) samples

According to the *E. coli* DNA spike concentration in ERC samples (Take the samples containing 30 pg of *E. coli* 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 the "ERC sample". The "ERC sample" should be processed in the same procedures as the test sample preparation before testing.

- Preparation of Negative Control Samples (NCS)

  Add 100 µL of DDB to a new 1.5 mL microcentrifuge tube, and label it as Negative Control Sample (NCS). The NCS should be processed in the same procedures as test sample preparation before testing.

## ■ qPCR Reaction MIX preparation

1. After thoroughly mixing *E. coli* qPCR MIX, follow 20 µL each tube is divided into 8-strip PCR tubes or 96-well plate.

2. Prepare qPCR Reaction MIX according to Table 3 and 96-well plate layouts as shown in Table 4.

Table 3. qPCR Reaction MIX preparation

| | |
|---|---|
| Standard curve | 20 µL *E. coli* qPCR MIX + 10 µL ST1/ST2/ST3/ST4/ ST5 |
| NTC | 20 µL *E. coli* qPCR MIX + 10 µL DDB |
| NCS | 20 µL *E. coli* qPCR MIX + 10 µL purified NCS |
| Test sample | 20 µL *E. coli* qPCR MIX + 10 µL purified test sample |
| ERC sample | 20 µL *E. coli* qPCR MIX + 10 µL purified ERC sample |

Table 4. 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.*

3. Seal the 96-well plate with sealing film. Mix it well in microplate shaker, then spin down the reagents for 10 seconds in microcentrifuge 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**, then enter *E. coli*-DNA in the Name field.
4. Select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
5. Add *E. coli*-DNA to **Detectors in Document**.
6. Select **ROX** as the passive reference dye, then Click **Next**.
7. Select the applicable set of wells for the samples, then select *E. coli*-DNA detector for each well.
8. Select **Finish**, and then set thermal-cycling conditions:
   a. Choose the thermal cycling reaction volume to 30μL.
   b. Set up the program as following:

Table 5. 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.

9. 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 6. 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 0.02.
   c. Select **Automatic Baseline**.
6. Click the button ▶ in the toolbar, then wait for the plate analysis to complete.
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 rate of ERC samples is calculated based on the results of the test samples and the ERC samples. The recovery rate 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.
12. The Ct value of NTC should not be lower than 35.00 or undetermined.

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

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## Support & Contact

SHENTEK

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189