SHENTEK

# Residual *E. coli* DNA Quantitation Kit

# User Guide

Version: A/1
For Research Use Only
Product No.: SK030202E100
Reagents for 100 Reactions

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*(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 uses quantitative PCR to perform 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* Control DNA, 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).

## ■ 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 |
|---|---|---|---|
| *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℃ |

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

- LineGene 9600plus Real-Time PCR System
- Mx3000PTM Real-Time PCR System
- qTOWER3G Real-Time PCR System

## ■ Required materials not included in the kit

- Nonstick, RNase-free microcentrifuge tubes and 1.5mL
- PCR 8-well strip tubes with caps or 96-well plate with seals
- Low retention filter tips: 1000 μL, 100 μL and 10 μL

## ■ Related equipment

- Real-Time PCR System
- Vortex mixer
- Benchtop microcentrifuge
- Pipettes: 1000 μL, 100 μL and 10 μL
- Microplate and microtube shaker

## ■ Workflow

Serial dilutions of the control DNA preparation

⇩

Sample preparation

⇩

qPCR reaction mix preparation

⇩

qPCR amplification

⇩

Results analysis

# 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.

## ■ *E. coli* 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.

Prepare a series of *E. coli* DNA Control solution with DNA Dilution Buffer (DDB) as follows:

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 centrifuge tubes as ST0, ST1, ST2, ST3, ST4 and ST5, respectively.
3. Transfer an 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.
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 |

- *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

➢ Preparation of Extraction Reference 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 example), the specific preparation procedure is as follows:

1. Aliquot 100 μL of the test sample to a new 1.5 mL centrifuge tube.

2. Add 10 μL of ST3 solution and mix thoroughly, label 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 centrifuge tube, and label 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 PCR 8-well strip tubes or 96-well plate .

2. Prepare qPCR Reaction MIX according to Table 3 and 96-well plate layout 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 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, and 5 test samples (S1 to S5), and 5 ERC sample (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.*

3. Seal the 96-well plate with sealing film. Mix it well in microplate shaker, then spin down the reagents for 10 seconds in centrifuge 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 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. 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 larger than the mean Ct value of the lowest concentration in the standard curve.
12. The Ct value of NTC should be Undetermined or no less than 35.00.

*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

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