

**Residual *E. coli* RNA  
Quantitation Kit (2G)  
User Guide**

Version: A/0

For Research use only

Product No.: 1201201-1

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* RNA Quantitation Kit (2G) is used for quantitation of host cell RNA from *E. coli* in a variety of biopharmaceutical products. This kit employs duplex reverse transcription quantitative PCR technique to performs a rapid, specific, and reliable quantitative detection of residual *E. coli* RNA. The target gene (FAM) can be determined at the femtogram (fg) level, whereas IPC-Internal Positive Control (VIC) is also included in the *E. coli* RNA Primer&Probe MIX to evaluate the performance of each PCR reaction.

### ■ Kit contents and storage

*WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions.*

Table 1. Kit components and storage

| Reagent  | Part No. | Quantity         | Storage                      |
|--|----------|------------------|------------------------------|
| <i>E.coli</i> RNA Control                        | NNA011   | 50 µL × 1 tube   | -20°C                        |
| One Step qPCR Buffer                             | NNB008   | 500 µL × 2 tubes | -20°C,<br>protect from light |
| One Step Enzyme MIX                              | NNC052   | 100 µL × 1 tube  | -20°C,<br>protect from light |
| <i>E.coli</i> RNA Primer&Probe MIX<br>(Incl IPC) | NNC119   | 400 µL × 1 tube  | -20°C,<br>protect from light |
| RNase-Free H <sub>2</sub> O                      | NND008   | 1.2 mL × 3 tubes | -20°C                        |

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 (Please validate the sensitivity before use)

- SHENTEK-96S Real-Time PCR System
- 7500 Real-Time PCR System
- Lightcycler 480 II Real-Time PCR System
- CFX96 Real-Time PCR System

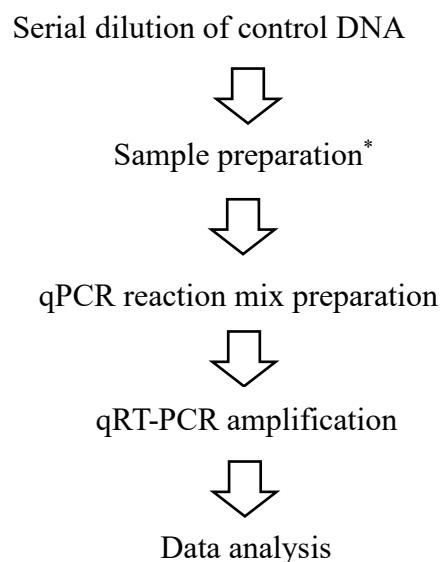
## ■ Required materials not included in the kit

- Nonstick, RNase-free, Low Retention Microfuge Tubes, 1.5mL
- Nonstick, Low Retention Tips 1000 µL, 100 µL, 10 µL
- DNase I and Buffer
- RNase inhibitor (Optional)
- 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps
- SHENTEK™ Residual Host Cell RNA Sample Preparation Kit (Product No. 1201205, Optional)

## ■ Related equipment

- Mini Centrifuge
- Vortex mixer
- Real-Time PCR System
- Pipettes 1000 µL, 100 µL, 10 µL
- Dry bath incubator
- Real-time PCR instrument

## ■ Workflow



*\*Please refer to SHENTEK™ Residual Host Cell RNA Sample Preparation Kit User Guide (Product No. 1201205).*

## 2. Methods

### ■ Experiment preparation

1. Wear appropriate protective eyewear, mask, clothing and gloves.
2. Irradiate the tabletop, micropipettes 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.

### ■ Dilution of *E. coli* RNA Control and preparation of standard curve

Please check the concentration on the label of *E. coli* RNA Control tube prior to dilution.

1. Thaw *E. coli* RNA Control and RNase-Free H<sub>2</sub>O completely at 2-8 °C or melt on ice. Flick the *E. coli* RNA Control tube gently, and briefly centrifuge 3-5 s, and repeat 3 times to mix well.
2. Label seven nonstick 1.5mL microfuge tubes: A, B, ST1, ST2, ST3, ST4 and ST5, respectively.
3. Transfer certain amount of RNase-Free H<sub>2</sub>O and *E. coli* RNA Control to A tube to achieve a 2 ng/μL control solution. Vortex and centrifuge briefly, and repeat for 3 times.
4. Add 45 μL RNase-Free H<sub>2</sub>O to each tube of B, ST1, ST2, ST3, ST4 and ST5.
5. Perform the serial dilutions according to Table 2:
  - a. Transfer 5 μL of RNA from tube A to B, then vortex for 5 seconds and spin for 5 seconds. Repeat 3 times to mix thoroughly.
  - b. Continue to transfer 5 μL of RNA to the next dilution tube until ST5.

Table 2. Dilution for *E. coli* RNA Control

| Serial dilution tube | Dilution  | Conc. (pg/μL) |
|----------------------|---|---------------|
| A                    | Dilute the RNA control with RNase-Free H <sub>2</sub> O | 2000          |
| B                    | 5 μL A + 45 μL RNase-Free H <sub>2</sub> O              | 200           |
| ST1                  | 5 μL B + 45 μL RNase-Free H <sub>2</sub> O              | 20            |
| ST2                  | 5 μL ST1 + 45 μL RNase-Free H <sub>2</sub> O            | 2             |
| ST3                  | 5 μL ST2 + 45 μL RNase-Free H <sub>2</sub> O            | 0.2           |
| ST4                  | 5 μL ST3 + 45 μL RNase-Free H <sub>2</sub> O            | 0.02          |
| ST5                  | 5 μL ST4 + 45 μL RNase-Free H <sub>2</sub> O            | 0.002         |

- The remaining, unused RNase-Free H<sub>2</sub>O can be stored at 2-8°C. If not used for a long time, please store at -20°C
- If the solution is cloudy or contains precipitates, heat at 37°C until it clears.
- It is recommended to include at least five concentration points for the standard curve, which can be selected based on the method validation data.

## ■ Sample preparation

According to different sample types, two different preparation ways are provided as below:

### ➤ Plasmid DNA samples derived from *E. coli* host bacteria

1. Apply DNase I treatment to the test samples, ERC, and NCS to eliminate the interference of genomic DNA (gDNA) on the detection. Follow the DNase digestion method outlined in Table 3:

Table 3. DNase Digestion of samples

| Reagents to add<br>in one sample  | Quantity required for each sample                 |                               |   |                       |
|---|---|-------------------------------|---|-----------------------|
|   | NCS (μL) <sup>(2)</sup><br>(Choose option A or B) |                               | Sample (μL)                                   | ERC<br>Sample<br>(μL) |
|   | Option A:<br>RNase-Free<br>H <sub>2</sub> O       | Option B:<br>Sample<br>matrix |   |                       |
| 10×DNase I Buffer   | 2   | 2                             | 2   | 2                     |
| DNase I (5 U/μL) <sup>(3)</sup>   | 2   | 2                             | 2   | 2                     |
| Sample or Sample matrix   | 0   | 2<br>(samples<br>matrix)      | 2 <sup>(3)</sup><br>(no more than<br>1 mg/μL) | 2                     |
| <i>E. coli</i> RNA Control  | 0   | 0                             | 0   | 5 <sup>(1)</sup>      |
| RNase inhibitor (Optional) <sup>(4)</sup>   | final concentration 0.2-1U/μL                     |                               |   |                       |
| Add RNase-Free H <sub>2</sub> O to  | 20 μL   |                               |   |                       |
| Incubate each sample at 37°C for 30 - 60 minutes for digestion.<br>(The digestion conditions are determined based on actual experience) |   |                               |   |                       |

*Note:*

(1) *Sample extraction recovery control (ERC):* In principle, the ERC Sample should ideally have an actual *E. coli* RNA concentration that is 2-10 times higher than the detected value in the sample. If the *E. coli* RNA concentration in the sample is lower than the LOQ of this kit, the spiked quantity should be adjusted within the assay range to

*ensure the accurate results.*

*(2) You can choose either sample matrix or RNase-Free H<sub>2</sub>O for NCS.*

*(3) Ensure that the final concentration of plasmid sample does not exceed 100 ng/μL, which requires the final concentration of DNase I at 0.2-2U/μL.*

*(4) Add RNase inhibitor to the digestion reaction can help to mitigate the potential impact of RNase from samples, consumables, environment, etc.*

2. Choose an appropriate DNase I inactivation method (one out of three methods below):

**Method 1:** Use SHENTEK™ Residual Host Cell RNA Sample Preparation Kit to treat the digestion solution of test samples, samples ERC, and NCS.

- *This inactivation method can effectively purify the sample matrix or digestion reaction solution, eliminating any potential matrix effects.*

**Method 2:** Inactivate DNase I at 75°C for 10 min.

**Method 3:** Use some other validated DNase inactivation or removal methods.

➤ **Samples from protein expression products using *E. coli* host bacteria**

1. Sample RNA Extraction

Use SHENTEK™ Residual Host Cell RNA Sample Preparation Kit to purify the test samples, ERC, and NCS.

2. Digest the extracted RNA samples from test samples, ERC sample, and NCS using DNase I to eliminate the potential influence of gDNA on the detection. Treat the digestion reactions according to Table 3.

3. Inactivate DNase I by heating the sample at 75°C for 10 min.

■ **qRT-PCR Mix preparation**

1. Prepare qRT-PCR MIX according to Table 4:

Table 4. qRT-PCR MIX Preparation

| Reagents                                       | Volume/well | Volume for 48 reactions (includes 10% overage) |
|--|-------------|--|
| One Step qPCR Buffer                           | 10 μL       | 530 μL   |
| One Step Enzyme MIX                            | 1 μL        | 53 μL  |
| <i>E. coli</i> RNA Primer&Probe MIX (Incl IPC) | 4 μL        | 212 μL   |
| Total  | 15 μL       | 795 μL   |

2. Vortex to mix well, and add 15 μL/well of qRT-PCR MIX to 96-well qPCR plates or

8-strip PCR tubes.

## ■ qRT-PCR reaction mix preparation

1. Prepare qRT-PCR Reaction mix as shown in Table 5:

Table 5. qRT-PCR Reaction mix preparation

| Reagent      | Standard curve                   | NTC                                    | NCS         | Test sample            | ERC sample         |
|--------------|----------------------------------|--|-------------|------------------------|--------------------|
| qRT-PCR MIX  | 15 µL                            | 15 µL                                  | 15 µL       | 15 µL                  | 15 µL              |
| Sample       | 5 µL<br>ST1/ST2/ST3/<br>ST4/ ST5 | 5 µL<br>RNase-Free<br>H <sub>2</sub> O | 5 µL<br>NCS | 5 µL<br>Test<br>sample | 5 µL<br>ERC Sample |
| Total Volume | 20 µL                            | 20 µL                                  | 20 µL       | 20 µL                  | 20 µL              |

## ■ qRT-PCR plate preparation

1. For the layout of the 96-well PCR plates, refer to Table 6.

Table 6. Plate layout of 96-well PCR reaction for example

|     |     |     |   |   |   |   |   |   |        |        |        |   |
|-----|-----|-----|---|---|---|---|---|---|--------|--------|--------|---|
| ST1 | ST1 | ST1 |   |   |   |   |   |   | S1     | S1     | S1     | A |
| ST2 | ST2 | ST2 |   |   |   |   |   |   | S2     | S2     | S2     | B |
| ST3 | ST3 | ST3 |   |   |   |   |   |   | S3     | S3     | S3     | C |
| ST4 | ST4 | ST4 |   |   |   |   |   |   |        |        |        | D |
| ST5 | ST5 | ST5 |   |   |   |   |   |   | S1-ERC | S1-ERC | S1-ERC | E |
|     |     |     |   |   |   |   |   |   | S2-ERC | S2-ERC | S2-ERC | F |
| NTC | NTC | NTC |   |   |   |   |   |   | S3-ERC | S3-ERC | S3-ERC | G |
| NCS | NCS | NCS |   |   |   |   |   |   |        |        |        | 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 no template control (NTC), 1 negative control sample (NCS), and 3 unknown test samples (S1 to S3), and 3 ERC samples (S1 ERC to S3 ERC), and 3 replicates are recommended 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 6.

2. Seal the 96-well plate with sealing film. Mix it well in microplate shaker, then spin down the reagents for 10 seconds in a centrifuge and place it onto the qPCR instrument.

## ■ qRT-PCR program setting

*NOTE: The following instructions apply only to the Applied Biosystems® 7500 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 *E. coli*-RNA 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 **Creat 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 *E. coli*-RNA detector and IPC detector for each well.
7. Select **Finish**, and then set thermal-cycling conditions:
  - a. Set the thermal cycling reaction volume to 20 µL.
  - b. Set the RT-qPCR program as following:

| Step                  | Temp. | Time(mm:sec) | Cycles |
|-----------------------|-------|--------------|--------|
| Reverse transcription | 50°C  | 15 :00       | 1      |
| Activation            | 95°C  | 00 :30       | 1      |
| Denature              | 95°C  | 00 :10       | 45     |
| Anneal/extend         | 60°C  | 00 :40       |        |

8. Save the document, then click **Start** to start the qRT-PCR 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 RNA detector task = **NTC**
  - b. NCS, test samples, and ERC wells: target RNA detector task = **Unknown**
2. Set up the standard curve as shown in the following table:

Table 9. Settings for Standard curve

| Tube label | Task     | Conc. (pg/reaction) |
|------------|----------|---------------------|
| ST1        | Standard | 100                 |
| ST2        | Standard | 10                  |
| ST3        | Standard | 1                   |
| ST4        | Standard | 0.1                 |
| ST5        | Standard | 0.01                |

3. In the Analysis Settings window, enter the following settings:
  - a. Select **Manual Ct**.
  - b. In the **Threshold field**, enter **0.05**.
  - c. Select **Automatic Baseline**.
4. Click the button  in the toolbar, then wait the plate analyzing.
5. Select the **Result** tab > > **Standard curve** tab, then verify the Slope, Intercept and R<sup>2</sup> values.
6. Select the **Report** tab, then achieve the mean quantity and standard deviation for each sample.
7. Select **File** > > **Export** > > **Results**. In the Save as type drop-down list, select **Results Export Files**, then click **Save**.
8. In the Report panel, the 'Mean Quantity' column shows the detection values of NTC, NCS, test sample, and sample ERC, in pg/Reaction.
9. The average Ct value of NTC should be at least 2 cycles higher than the lowest standard curve.
10. The recovery rate of ERC samples are calculated based on the results of the test samples and the ERC samples. The recovery rates should be between 50% and 150%.

11. If the Ct -IPC value of the sample is significantly larger than the Ct-IPC value of NTC or NCS, it indicates that the reaction may be inhibited by test sample. Please consider sample recovery rate prior to IPC results, and IPC results can only be used as reference.

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

## ■ Related products

| Product No. | Product Name   | Quantity      |
|-------------|--|---------------|
| 1201205     | SHENTEK™ Residual Host Cell RNA Sample Preparation Kit | 100 Reactions |

Effective date: 26 Feb. 2024

## Support & Contact



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