SHENTEK

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

User Guide

Version: A/2
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 perform 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. Wear appropriate protective eyewear, mask, clothing and gloves.

Table 1. Kit components and storage

Reagent	Part No.	Quantity	Storage
E. coli RNA Control	NNA011	50 μL × 1 tube	-20℃
One Step qPCR Buffer	NNB008	500 μL × 2 tubes	-20℃, protect from light
One Step Enzyme MIX	NNC052	100 μL × 1 tube	-20℃, protect from light
E. coli RNA Primer&Probe MIX (Incl IPC)	NNC119	400 μL × 1 tube	-20℃, protect from light
RNase-Free H_2O	NND008	1.2 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 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
- Lightcycler 480 II Real-Time PCR System
- CFX96 Real-Time PCR System

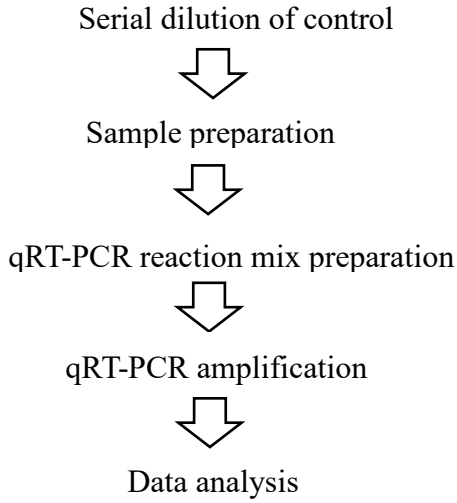
■ Required materials not included in the kit

- Low retention, RNase/DNase-free, sterile microcentrifuge tubes
- Low retention filter tips: 1000 μL, 100 μL and 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

- Benchtop microcentrifuge
- Vortex mixer
- Real-Time PCR System
- Pipettes: 1000 μL, 100 μL, 10 μL
- Dry bath incubator
- Microplate 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.

■ *E. coli* RNA Control serial dilutions for the 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_2O completely at 2-8°C or melt on ice. Flick the *E. coli* RNA Control tube gently, and briefly centrifuge 3-5 seconds, and repeat 3 times to mix well.
2. Label 1.5 mL microcentrifuge tubes: A, B, ST1, ST2, ST3, ST4 and ST5, respectively.
3. Transfer certain amount of RNase-Free H_2O and *E. coli* RNA Control to A tube to achieve a 2000 pg/μL control solution. Vortex and centrifuge briefly, and repeat for 3 times.

$$\frac{\text{RNA Control conc. (A)}\times 1000\text{ pg/ng}\times\text{Volume of RNA Control (B)}}{2000\text{ pg/µL}}-\text{Volume of RNA Control (B)}$$

For example:

The concentration on the label of the RNA Control is 20.4 ng/µL (A), pipette 5 µL (B) of the RNA Control to the A tube. Add the calculated volume below to reach 2000 pg/µL.

$$\frac{20.4\text{ ng/µL}\times 1000\text{ pg/ng}\times 5\text{ µL}}{2000\text{ pg/µL}}-5\text{ µL}=46\text{ µL}$$

4. Add 45 µL RNase-Free H_2O 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_2O	2000
B	5 µL A + 45 µL RNase-Free H_2O	200
ST1	5 µL B + 45 µL RNase-Free H_2O	20
ST2	5 µL ST1 + 45 µL RNase-Free H_2O	2
ST3	5 µL ST2 + 45 µL RNase-Free H_2O	0.2
ST4	5 µL ST3 + 45 µL RNase-Free H_2O	0.02
ST5	5 µL ST4 + 45 µL RNase-Free H_2O	0.002

- *The remaining unused RNase-Free H_2O can be stored at 2-8℃. If not used for a long time, please store at -20℃. If the solution is cloudy or contains precipitates, heat at 37℃ until it clear.*
- *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 in one sample	**Quantity required for each sample**			
	NCS (μL)(2) (Choose option A or B)		**Sample (μL)**	**ERC Sample (μL)**
	Option A: RNase-Free H_2O	**Option B: Sample matrix**		
10×DNase I Buffer	2	2	2	2
DNase I (5 U/μL)(3)	2	2	2	2
Sample or Sample matrix	0	2 (samples matrix)	2(3) (no more than 1 mg/μL)	2
E. coli RNA Control	0	0	0	5(1)
RNase inhibitor (Optional)(4)	final concentration 0.2-1U/μL			
Add RNase-Free H_2O to	20 μL			
Incubate each sample at 37℃ for 30 - 60 minutes for digestion. (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 QL 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_2O for NCS.

(3) Ensure that the final concentration of plasmid sample does not exceed 100

ng/uL, 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℃ for 10 minutes.

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℃ 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
E. coli 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 ST1/ST2/ST3 /ST4/ ST5	5 μL RNase-Free H_2O	5 μL NCS	5 μL Test sample	5 μL 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:

Table 7. qPCR running temperature and time

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

*Instrument will read the fluorescence signal during this step.

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 8. Settings for Standard curve

Tube label	Task	Conc. (pg/μL)
ST1	Standard	20
ST2	Standard	2
ST3	Standard	0.2
ST4	Standard	0.02
ST5	Standard	0.002

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^2 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 Qty' column shows the detection values of NTC, NCS, test sample, and sample ERC, in pg/μL.
9. The average Ct value of NTC should be at least 2 cycles higher than the lowest standard curve.
10. The recovery of ERC samples are calculated based on the results of the test samples and the ERC samples. The recovery 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 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.

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Support & Contact

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