NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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SHENTEK

Residual 293T RNA Quantitation Kit
User Guide

Version: A/0
For Research Use Only
Product No.: 1201202
Reagents for 100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual 293T RNA Quantitation Kit is used to quantitate residual 293T cell total RNA in different stages of biopharmaceutical products, from in-process samples to final products. Through the designing of specific primers and probes, the reverse transcription and fluorescence quantitative PCR detection techniques were employed to achieve a one-step quantitative detection of residual total RNA. This kit performs a rapid, specific, and reliable quantitation assay at the femtogram (fg) level. IPC (Internal Positive Control) is included in the kit to evaluate the performance of each PCR reaction. For extraction information, please refer to the SHENTEK® Residual Host Cell RNA Sample Preparation Kit User Guide (Product No. 1201205).

■ 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
293T RNA Control	NNA052	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	
293T RNA Primer&Probe MIX	NNC096	400 μL × 1 tube	
RNA IPC Primer&Probe MIX	NNC053	200 μL × 1 tube	
RNase-Free H_2O	NND008	1.2 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

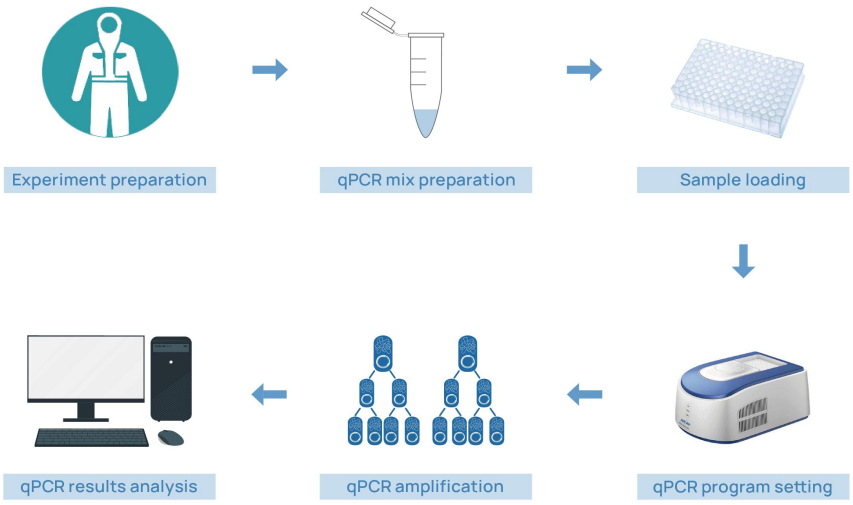
■ Required materials not included in the kit

- Nonstick, DNase-free & Low Retention Microfuge Tubes, 1.5 mL
- Nonstick, Low Retention Tips of 1000 µL, 100 µL, 10 µL
- 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps
- DNase and Buffer

■ Related equipment

- Real-Time PCR System
- Vortex mixer
- Microcentrifuge
- Microplate shaker
- Micropipettes of 1000 µL, 100 µL and 10 µL

■ Workflow



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, vortex and spin briefly.

■ 293T RNA Control serial dilutions for the standard curve

Please check the concentration labeled on the tube containing the 293T RNA Control prior to dilution.

1. Thaw 293T RNA Control and RNase-Free H_2O completely at 2-8°C or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times.
2. Label seven nonstick 1.5 mL microfuge tubes: ST, ST0, ST1, ST2, ST3, ST4 and ST5.
3. Dilute the 293T RNA Control to 2000 pg/μL with RNase-Free H_2O in the ST tube. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.
4. Add 45 μL RNase-Free H_2O to each tube of ST0, ST1, ST2, ST3, ST4, ST5.
5. Perform the serial dilutions according to Table 2:

Table 2. Dilution for 293T RNA Control

Serial dilution tube	Dilution	Conc. (pg/μL)
ST0	5 μL ST + 45 μL RNase-Free H_2O	200
ST1	5 μL ST0 + 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 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

➢ Test Sample Preparation

Take 100 μL of each test sample and add to a new 1.5 mL microfuge tube.

The sample to be tested should be treated with DNase before detection to eliminate the influence of gDNA on detection. The dosage and digestion conditions of DNase should be optimized according to the actual sample.

➢ Negative Control Sample (NCS) Preparation

Add 100 μL of RNase-Free H_2O to a new 1.5 mL microfuge tube, and label as NCS.

NCS and samples should be prepared in same way for DNA extraction.

■ qRT-PCR MIX preparation

1. Determine the number of reaction wells based on your selected standard curve, with the number of test samples and control samples. Generally, triplicates are tested for each sample.

Number of reaction wells = (5 standard points on the standard curve + 1 NTC + 1 NCS + test samples)×3

2. Prepare qRT-PCR MIX according to the number of reaction wells in Table 3.

Table 3. qRT-PCR MIX Preparation

Reagents	Volume/ reaction	Volume for 30 reaction (includes 10% overage)
One Step qPCR Buffer	10 μL	330 μL
One Step Enzyme MIX	1 μL	33 μL
293T RNA Primer&Probe MIX	4 μL	132 μL
Total volume	15 μL	495 μL

3. Mix thoroughly and place on ice, aliquot 15 μL/well into 96-well qPCR plate

or PCR 8-strip tubes.

■ IPC qRT-PCR MIX preparation

1. IPC of one negative control sample (IPC-NCS) and IPC of each test sample (IPC-S) should be tested in each experiment.

2. Prepare IPC qRT-PCR MIX according to the number of reaction wells in Table

Table 4. IPC qRT-PCR MIX Preparation

Reagents	Volume/ reaction	Volume for 6 reaction (includes 10% overage)
One Step qPCR Buffer	10 μL	66 μL
One Step Enzyme MIX	1 μL	6.6 μL
RNA IPC Primer&Probe MIX	4 μL	26.4 μL
Total volume	15 μL	99 μL

3. Mix thoroughly and place on ice, aliquot 15 μL/well into 96-well qPCR plate or PCR 8-strip tubes.

■ qRT-PCR Reaction MIX and IPC qRT-PCR Reaction MIX preparation

1. Prepare qRT-PCR Reaction MIX and IPC qRT-PCR Reaction MIX according to Table 5 and 6, and example of 96-well plate layout as shown in Table 7.

Table 5. qRT-PCR Reaction MIX Preparation

Tubes	Standard curve	NTC	NCS	Test sample
qRT-PCR MIX	15 μL	15 μL	15 μL	15 μL
Samples	5 μL ST1 - ST5	5 μL RNase-Free H_2O	5 μL purified NCS	5 μL purified test sample
Total Volume	20 μL	20 μL	20 μL	20 μL

Table 6. IPC qRT-PCR Reaction MIX Preparation

Tubes	NCS	Test sample
IPC qRT-PCR MIX	15 μL	15 μL
Samples	5 μL purified NCS	5 μL purified test sample
Total Volume	20 μL	20 μL

Table 7. Example of 96-well Plate layout

ST1	ST1	ST1										A
ST2	ST2	ST2										B
ST3	ST3	ST3							IPC-S1	IPC-S1	IPC-S1	C
ST4	ST4	ST4							IPC-S2	IPC-S2	IPC-S2	D
ST5	ST5	ST5							IPC-S3	IPC-S3	IPC-S3	E
				NTC	NTC	NTC			S1	S1	S1	F
				NCS	NCS	NCS			S2	S2	S2	G
				IPC-NCS	IPC-NCS	IPC-NCS			S3	S3	S3	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, 1 IPC for NCS (IPC-NCS), 3 test samples (S1 to S3), and 3 IPC for test samples (IPC-S1 to IPC-S3), with 3 replicates 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 7.*

2. 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 on the qPCR instrument.

■ qRT-PCR 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:**

a. Enter 293T-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 **Create Another**.

4. Click **New Detector:**

a. Enter RNA-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 293T-RNA detector and RNA-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 temperature and time as following (Table 8):

Table 8. qRT-PCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Reverse Transcription	50℃	15:00	1
Activation	95℃	00:30	1
Denaturation	95℃	00:10	45
Annealing/extension	60℃*	00:40	

*Instrument will read the fluorescence signal during this step.

8. Save the document, then click **Start** to start the real-time 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 DNA detector task = **NTC**

b. NCS and test samples wells: target DNA detector task = **Unknown**

2. Set up the standard curve as shown in table 9:

Table 9. Settings for Standard curve

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

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, 293T-RNA and RNA-IPC 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. In the Report panel of Results, the 'Mean Quantity' column shows the detection values of NTC, NCS and test sample, in pg/μL.
11. The Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve, and shows normal amplification curve in the VIC signal channel.
12. The Ct value of NTC should be 2 cycles greater than the mean of the lowest concentration in the standard curve.

13. The Ct-IPC value of the sample and the Ct-IPC value of the NCS should be within the range of ±1.0 Ct value. If the Ct-IPC value of the sample is significantly higher than the Ct-IPC value of the NCS, it indicates that the sample may be inhibited. If the samples that include 293T RNA spike concentration are tested at the same time, please consider sample recovery rates as priority, and take the IPC results as a reference.

Note: The parameter settings of the result analysis should be configured 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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