NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

SHENTEK Biofargo

www.biofargo.com
804-529-2296
contact@biofargo.com

SHENTEK

Residual MRC-5 DNA Quantitation Kit User Guide

Version: A/0
For Research Use Only
Product No.: 1101124
Reagents for 100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual MRC-5 DNA Quantitation Kit is used to quantitate residual MRC-5 host cell DNA in different stages of biopharmaceutical products, from in-process samples to final products. This kit utilizes quantitative PCR (qPCR) technique to perform a rapid, specific, and reliable quantitation assay at the femtogram (fg) level. The kit provides MRC-5 DNA Control as reference standard. For extraction information, please refer to the SHENTEK® Residual Host Cell DNA Sample Preparation Kit User Guide (Product No. 1104191).

■ 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
MRC-5 DNA Control	NNA062	50 µL × 1 tube	-20℃
qPCR Reaction Buffer	NNB001	850 µL × 2 tubes	-20℃, protect from light
MRC-5 Primer&Probe MIX	NNC118	300 µL × 1 tube	
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
- ABI 7500 Real-Time PCR System

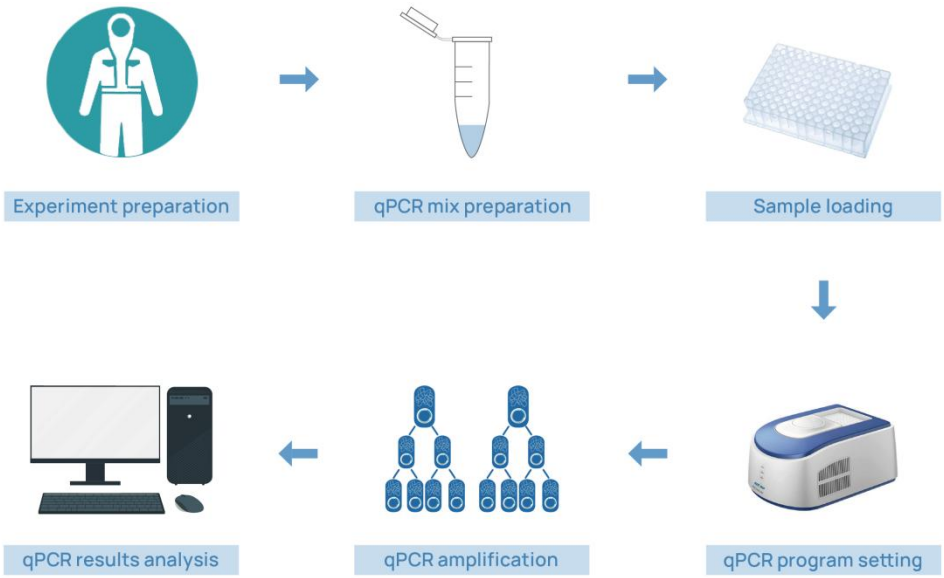
■ Required materials not included in the kit

- Low retention, RNase/DNase-free, sterile microcentrifuge tubes
- 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
- Benchtop microcentrifuge
- Vortex mixer
- 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.

■ MRC-5 DNA Control serial dilutions for the standard curve

Please check the concentration labeled on the tube containing the MRC-5 DNA Control prior to dilution.

1. Thaw MRC-5 DNA Control and DNA Dilution Buffer (DDB) 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 six 1.5 mL microcentrifuge tubes: ST0, ST1, ST2, ST3, ST4 and ST5.
3. Dilute the MRC-5 DNA Control to 3000 pg/μL with DDB in the ST0 tube. Calculate the volume of DDB to prepare the ST0:

$$\frac{\text{DNA Control conc. (A)} \times 1000\ \text{pg/ng} \times \text{Volume of DNA Control (B)}}{3000\ \text{pg/uL}} - \text{Volume of DNA Control (B)}$$

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 the below volume to reach 3000 pg/uL.

$$\frac{30.9\ \text{ng/uL} \times 1000\ \text{pg/ng} \times 10\ \text{uL}}{3000\ \text{pg/uL}} - 10\ \text{uL} = 93\ \text{uL}$$

4. Vortex to mix well and quickly spin down the ST0 tube for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.
5. Add 90 μL DDB to each tube of ST1, ST2, ST3, ST4 and ST5.
6. Perform the serial dilutions according to Table 2:

Table 2. Dilution for MRC-5 DNA Control

Serial dilution tube	Dilution	Conc. (pg/μL)
ST0	Dilute the DNA Control with DDB	3000
ST1	10 μL ST0 + 90 μL DDB	300
ST2	10 μL ST1 + 90 μL DDB	30
ST3	10 μL ST2 + 90 μL DDB	3
ST4	10 μL ST3 + 90 μL DDB	0.3
ST5	10 μL ST4 + 90 μL DDB	0.03

- *The remaining unused DDB needs 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 performing method validation before sample testing.*

■ Sample preparation

➢ Extraction Reference Control (ERC) Sample Preparation

According to the MRC-5 DNA spike concentration in ERC samples (take the sample containing 30 pg of MRC-5 DNA as example), specific preparation procedure is as follows:

(1) Take 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 as ERC sample.

➢ Negative Control Sample (NCS) Preparation

Add 100 μL of DDB to a new 1.5 mL microcentrifuge tube, and label as NCS.

ERC, NCS should be processed in the same procedures as test sample preparation before testing.

■ qPCR MIX preparation

1. Determine the number of reaction wells based on the 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 qPCR MIX according to the number of reaction wells in Table 3.

Table 3. qPCR MIX preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	17 μL	561 μL
MRC-5 Primer&Probe MIX	3 μL	99 μL
Total volume	20 μL	660 μL

3. Mix thoroughly and place on ice, aliquot 20 μL/well into 96-well qPCR plate or PCR 8-strip tubes.

■ qPCR Reaction MIX preparation

1. Prepare qPCR Reaction MIX according to Table 4, and 96-well plate layout is shown in Table 5.

Table 4. qPCR Reaction MIX preparation

Tubes	Volume/reaction
Standard curve	20 μL qPCR MIX + 10 μL ST1/ST2/ST3/ST4/ ST5
NTC	20 μL qPCR MIX + 10 μL DDB
NCS	20 μL qPCR MIX + 10 μL Extracted NCS
Test sample	20 μL qPCR MIX + 10 μL Extracted test sample
ERC sample	20 μL qPCR MIX + 10 μL Extracted ERC sample

Table 5. Example of 96-well Plate layout

NTC		S1	S1	S1	S1 ERC	S1 ERC	S1 ERC					A
NTC		S2	S2	S2	S2 ERC	S2 ERC	S2 ERC		ST5	ST5	ST5	B
NTC		S3	S3	S3	S3 ERC	S3 ERC	S3 ERC		ST4	ST4	ST4	C
		S4	S4	S4	S4 ERC	S4 ERC	S4 ERC		ST3	ST3	ST3	D
NCS		S5	S5	S5	S5 ERC	S5 ERC	S5 ERC		ST2	ST2	ST2	E
NCS									ST1	ST1	ST1	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, 5 test samples (S1 to S5), and 5 ERC samples (S1 ERC to S5 ERC), 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 5.*

2. Seal the 96-well plate with sealing film. Mix well in microplate shaker, then spin down the reagents for 10 seconds in microcentrifuge and place it on 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:**
   a. Enter MRC-5-DNA 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. Select **ROX** as the passive reference dye, then Click **Next**.
5. Select the applicable set of wells for the samples, then select MRC-5-DNA detector for each well.
6. Select Finish, and then set thermal-cycling conditions:
   a. Set the thermal cycling reaction volume to 30 μL.
   b. Set the temperature and time as follow in Table 6:

Table 6. 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.

7. 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 table 7:

Table 7. Settings for Standard curve

Tube label	Task	Quantity (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, MRC-5-DNA 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, test sample, and ERC sample, in pg/μL.
11. The recovery rate of ERC samples is calculated based on the value of the test

samples and the ERC samples. The recovery rates should be between 50% and 150%.

12. 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.
13. The detection value of NTC should be no more than 6.00 fg/μL, or set specific criteria by your own method validation, meanwhile shows normal amplification curve in the VIC signal channel.

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

SHENTEK

Biofargo, Inc.
www.biofargo.com
Address: 1716 E Parham Rd Richmond, Va, 23228, USA
E-mail: contact@biofargo.com
Phone: 804-529-2296