NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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SHENTEK

Residual Sf9 DNA Size Analysis Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1103177
Reagents for 4×100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual Sf9 DNA Size Analysis Kit is used to quantitate fragment sizes of residual Sf9 DNA in different stages of biopharmaceutical products, from in-process samples to final products.

This kit utilizes real-time PCR technique to perform rapid and specific quantitation of residual Sf9 DNA fragments (FAM) in samples. It is designed to amplify four different fragments (87bp, 118bp, 225bp and 500bp) for the accurate determination of their size distribution at LOD level of femtograms (fg). 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
Sf9 DNA Control	NNA033	50 µL × 1 tube	-20℃
qPCR Reaction Buffer	NNB002	850 µL × 8 tubes	-20℃, protect from light
Sf9 Primer&Probe MIX-87	NNC060	300 µL × 1 tube	
Sf9 Primer&Probe MIX-118	NNC061	300 µL × 1 tube	
Sf9 Primer&Probe MIX-225	NNC062	300 µL × 1 tube	
Sf9 Primer&Probe MIX-500	NNC063	300 µL × 1 tube	
IPC MIX	NNC069	550 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
- LightCycler 480II Real-Time PCR System

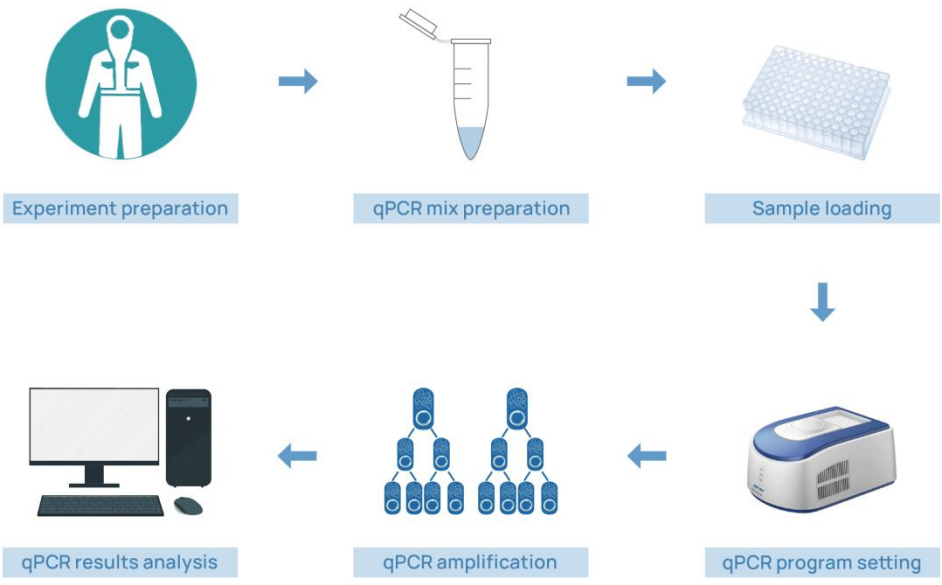
■ Required materials not included in the kit

- 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 equipments

- 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.

■ DNA Control serial dilutions for the standard curve

Note: The kit contains four Sf9 primer & probe mixes for different fragment lengths. Please set up four separate standard curves corresponding to each fragment length.

Please check the concentration labeled on the tube containing the Sf9 DNA Control prior to dilution.

Prepare four sets of Sf9 DNA Control solution with DNA Dilution Buffer (DDB) following the serial dilution procedure below:

1. Thaw Sf9 DNA Control and 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 seven 1.5 mL microcentrifuge tubes: ST0, ST1, ST2, ST3, ST4, ST5 and ST6.
3. Dilute the 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 180 μL DDB to each tube of ST1, ST2, ST3, ST4, ST5 and ST6.
6. Perform the serial dilutions according to Table 2:

Table 2. Dilution for Sf9 DNA Control

Serial dilution tube	Dilution	Conc. (pg/μL)
ST0	Dilute the DNA Control with DDB	3000
ST1	20 μL ST0+180 μL DDB	300
ST2	20 μL ST1+180 μL DDB	30
ST3	20 μL ST2+180 μL DDB	3
ST4	20 μL ST3+180 μL DDB	0.3
ST5	20 μL ST4+180 μL DDB	0.03
ST6	20 μL ST5+180 μL DDB	0.003

- *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 concentrations of standard curve should be included. To select appropriate sample dilutions, we recommend performing method validation before sample testing.*

■ Sample preparation

➢ Negative Control Sample (NCS) Preparation

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

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 = (6 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 3-6.

Table 3. qPCR MIX-87 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
Sf9 Primer&Probe MIX-87	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 4. qPCR MIX-118 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
Sf9 Primer&Probe MIX-118	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 5. qPCR MIX-225 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
Sf9 Primer&Probe MIX-225	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 6. qPCR MIX-500 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
Sf9 Primer&Probe MIX-500	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

For simultaneous detection of the four fragments, please prepare at least 120 μL

template DNA for four assays.

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 7-10, and a 96-well plate layout template is shown in Table 11.

Table 7. qPCR Reaction MIX-87 preparation

Tubes	Standard curve	NTC	NCS	Test sample
qPCR MIX-87	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 8. qPCR Reaction MIX-118 preparation

Tubes	Standard curve	NTC	NCS	Test sample
qPCR MIX-118	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 9. qPCR Reaction MIX-225 preparation

Tubes	Standard curve	NTC	NCS	Test sample
qPCR MIX-225	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 10. qPCR Reaction MIX-500 Preparation

Tubes	Standard curve	NTC	NCS	Test sample
qPCR MIX-500	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST6	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 11. Example of 96-well plate layout

Plate 1:

MIX-87						MIX-118						
NTC	NTC	NTC				NTC	NTC	NTC				A
NCS	NCS	NCS				NCS	NCS	NCS				B
			ST6	ST6	ST6				ST6	ST6	ST6	C
			ST5	ST5	ST5				ST5	ST5	ST5	D
S1	S1	S1	ST4	ST4	ST4	S1	S1	S1	ST4	ST4	ST4	E
S2	S2	S2	ST3	ST3	ST3	S2	S2	S2	ST3	ST3	ST3	F
S3	S3	S3	ST2	ST2	ST2	S3	S3	S3	ST2	ST2	ST2	G
S4	S4	S4	ST1	ST1	ST1	S4	S4	S4	ST1	ST1	ST1	H
1	2	3	4	5	6	7	8	9	10	11	12	

Plate 2:

MIX-225						MIX-500						
NTC	NTC	NTC				NTC	NTC	NTC				A
NCS	NCS	NCS				NCS	NCS	NCS				B
			ST6	ST6	ST6				ST6	ST6	ST6	C
			ST5	ST5	ST5				ST5	ST5	ST5	D
S1	S1	S1	ST4	ST4	ST4	S1	S1	S1	ST4	ST4	ST4	E
S2	S2	S2	ST3	ST3	ST3	S2	S2	S2	ST3	ST3	ST3	F
S3	S3	S3	ST2	ST2	ST2	S3	S3	S3	ST2	ST2	ST2	G
S4	S4	S4	ST1	ST1	ST1	S4	S4	S4	ST1	ST1	ST1	H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents four assays, including selected standard curve points of Sf9 DNA Control (ST1-ST6),1 NTC,1 NCS, 4 test samples(S1-S4), and 3 replicates for each sample.*
- *The plate layout for sample loading can be adjusted based on the sample quantity.*

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 v2.4. 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. Click **New Detector**, then enter Sf9-87 in the Name field, select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.

3. Create new detector for Sf9-118, Sf9-225 and Sf9-500, separately as step2.

4. Click **New Detector**, then enter IPC in the Name field. Select **VIC** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.

5. Select **ROX** as the passive reference dye, then click **Next**.

6. Select the applicable set of wells for the samples, then select the corresponding detector for each well.

7. 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 following in Table 12:

Table 12. qPCR running temperature and time

Step	Temp.	Time(mm:sec)	Cycles
Activation	95℃	10:00	1
Denaturation	95℃	00:15	40
Annealing	60℃	00:30	
Extension	72℃*	01:30	

*Instrument will read the fluorescence signal during this step.

8. Save the document, then click **Start** to start the 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= **Unknown**

2. Set up the standard curve as shown in the following table 13:

Table 13. 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
ST6	Standard	0.003

Note:

On the 7500 Real-Time PCR System, the recommended standard concentrations of MIX-87 and MIX-118 is ST1 to ST6, while for MIX-225 and MIX-500, it is from ST1 to ST5.

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. VIC Threshold field, enter 0.1.

   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, 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**.

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.

10. Set the value of DNA size-87 to be 100%, calculate the percentage of the DNA size of 118, 225 and 500.

11. Analyze the Ct value of IPC. Normally, the mean Ct-IPC value of the sample should be within ±1.0 of the NCS Ct-IPC value. If the mean Ct-IPC value of the sample is significantly higher than the Ct-IPC value of the NCS, this indicates that the sample may be inhibitory to the assay. We recommend to test the ERC samples at the same assay, and take the sample recovery rate as the criterion.

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 Ct value of NTC should not be less than 35.00 cycles or undetermined, meanwhile shows normal amplification curve in the VIC signal channel.

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Support & Contact

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