NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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SHENTEK

Residual BHK DNA
Size Analysis Kit
User Guide

Version: A/0
For Research Use Only
Product No.: 1103179
Reagents for 4×100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual BHK DNA Size Analysis Kit is used to quantitate BHK DNA residues of different fragment sizes in different stages of biopharmaceutical products, from in-process samples to final products. This kit utilizes quantitative PCR (FAM) technique to perform rapid and specific quantitation of residual BHK DNA fragments in samples. It is designed to amplify four different fragments (81bp, 134bp, 216bp, 589bp) for the accurate size distribution, and at the limit of detection (LOD) reaches femtograms (fg) level. 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
BHK DNA Control	NNA029	50 µL × 1 tube	-20℃
qPCR Reaction Buffer	NNB001	850 µL × 8 tubes	-20℃, protect from light
BHK Primer&Probe MIX-81	NNC072	300 µL × 1 tube	
BHK Primer&Probe MIX-134	NNC073	300 µL × 1 tube	
BHK Primer&Probe MIX-216	NNC074	300 µL × 1 tube	
BHK Primer&Probe MIX-589	NNC075	300 µL × 1 tube	
IPC MIX	NNC070	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
- 7500 Real-Time PCR system
- Lightcycler 480 Real-Time PCR System

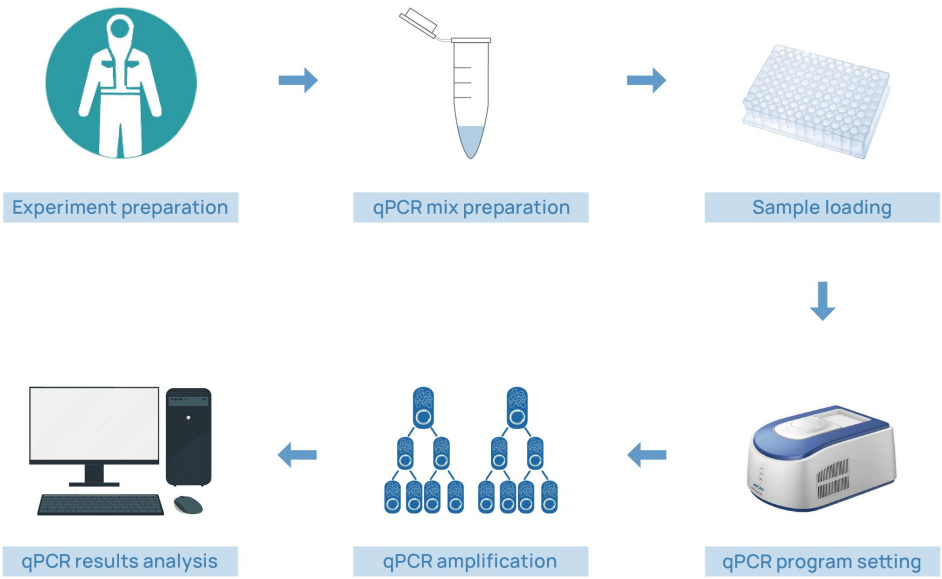
■ Required materials not included in the kit

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

■ Related equipments

- Real-Time PCR system
- Benchtop microcentrifuge
- Vortex mixer
- Micropipettes: 1000 µL, 100 µL and 10 µL

■ Workflow



2. Methods

■ Experiment preparation

1. Wear appropriate protective eyewear, clothing, mask and gloves.

2. Irradiate the tabletop, pipettes and tubes with UV for 30 minutes, and disinfect with 75% ethenol.

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 BHK primer & probe sets 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 BHK DNA Control prior to dilution.

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

1. Thaw BHK 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 six nonstick 1.5 mL microfuge tubes: ST0, ST1, ST2, ST3, ST4 and ST5.
3. Dilute the DNA Control to 3000 pg/μL with DDB in the ST0 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 180 μL DDB to each tube of ST1, ST2, ST3, ST4 and ST5.
5. Perform serial dilutions according to Table 2:

a. Transfer 20 μL of the DNA control from tube ST0 to ST1, then vortex to mix well and quickly spin down the reagents for 3-5 seconds in microcentrifuge. Repeat vortex and spin for 3 times.

b. Continue to transfer 20 μL of DNA control solution from the previous dilution

tube to the next until you reach tube ST5.

Table 2. Dilution for BHK 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

- *The remaining unused DDB 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*

■ Negative Control Sample (NCS) preparation

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

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

■ qPCR 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 = (standard curve of 5 concentration gradients + 1 NTC + 1 NCS + test samples) ×3

2．Prepare qPCR MIX according to Table 3, 4, 5 and 6.

Table 3. qPCR MIX-81 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
BHK Primer&Probe MIX-81	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 4. qPCR MIX-134 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
BHK Primer&Probe MIX-134	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 5. qPCR MIX-216 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
BHK Primer&Probe MIX-216	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 6. qPCR MIX-589 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
BHK Primer&Probe MIX-589	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 reactions following Table 7-10, and 96-well plate layout template is shown in Table 11.

Table 7. qPCR Reaction MIX-81 preparation

Tubes	ST-81	NTC	NCS	Test sample
qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST5	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-134 preparation

Tubes	ST-134	NTC	NCS	Test sample
qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST5	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-216 preparation

Tubes	ST-216	NTC	NCS	Test sample
qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST5	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-589 Preparation

Tubes	ST-589	NTC	NCS	Test sample
qPCR MIX	20 μL	20 μL	20 μL	20 μL
Samples	10 μL ST1 - ST5	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-81						MIX-134						
ST5	ST5	ST5	S1	S1	S1	ST5	ST5	ST5	S1	S1	S1	A
ST4	ST4	ST4	S2	S2	S2	ST4	ST4	ST4	S2	S2	S2	B
ST3	ST3	ST3	S3	S3	S3	ST3	ST3	ST3	S3	S3	S3	C
ST2	ST2	ST2	S4	S4	S4	ST2	ST2	ST2	S4	S4	S4	D
ST1	ST1	ST1				ST1	ST1	ST1				E
												F
												G
NTC	NTC	NTC	NCS	NCS	NCS	NTC	NTC	NTC	NCS	NCS	NCS	H
1	2	3	4	5	6	7	8	9	10	11	12	

Plate 2:

MIX-216						MIX-589						
ST5	ST5	ST5	S1	S1	S1	ST5	ST5	ST5	S1	S1	S1	A
ST4	ST4	ST4	S2	S2	S2	ST4	ST4	ST4	S2	S2	S2	B
ST3	ST3	ST3	S3	S3	S3	ST3	ST3	ST3	S3	S3	S3	C
ST2	ST2	ST2	S4	S4	S4	ST2	ST2	ST2	S4	S4	S4	D
ST1	ST1	ST1				ST1	ST1	ST1				E
												F
												G
NTC	NTC	NTC	NCS	NCS	NCS	NTC	NTC	NTC	NCS	NCS	NCS	H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents four assays, including selected standard curve points of BHK DNA Control (ST1-ST5),1 NTC,1 NCS and 4 test sample (S1-S4), with 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 centrifuge 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. Click **New Detector**, then enter BHK-81 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 BHK-134, BHK-216 and BHK-589, 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 follo 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

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.

   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. For test samples, set the value of DNA size-81 to be 100%, calculate the percentage of the DNA size of 134, 216 and 589.
11. The recovery rate of ERC samples is calculated based on the value of test

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

12. To analyze the Ct value of IPC, normally 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 consider the sample recovery rate as priority.

13. 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 VIC signal channel. If the validated limit of quantitation (LOQ) concentration is less than the lowest concentration in the standard curve, the value of the NCS should be less than the concentration of LOQ.

14. The Ct value of NTC should be no less than 35.00 cycles, meanwhile shows normal amplification curve in VIC signal channel.

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.

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