NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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SHENTEK

Residual Human DNA Size Analysis Kit (2G) User Guide

Version: A/1
For Research Use Only
Product No.: 1103173
Reagents for 4×100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual Human DNA Size Analysis Kit (2G) is used to quantitate Human DNA residues of different fragment sizes at varying 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 human DNA fragments in samples. It is designed to amplify four different fragments (75 bp, 122 bp, 244 bp and 562 bp) for the accurate determination of their size distribution with the 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
qPCR Reaction Buffer	NNB001	850 µL × 8 tubes	-20℃, protect from light
Human Primer&Probe MIX-75	NNC008	300 µL × 1 tube	
Human Primer&Probe MIX-122	NNC009	300 µL × 1 tube	
Human Primer&Probe MIX-244	NNC010	300 µL × 1 tube	
Human Primer&Probe MIX-562	NNC011	300 µL × 1 tube	
IPC MIX	NNC069	550 µL × 1 tube	
Human DNA Control	NNA003	50 µL × 1 tube	-20℃
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 3 tubes	

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
- FQD-96A Real-Time PCR system

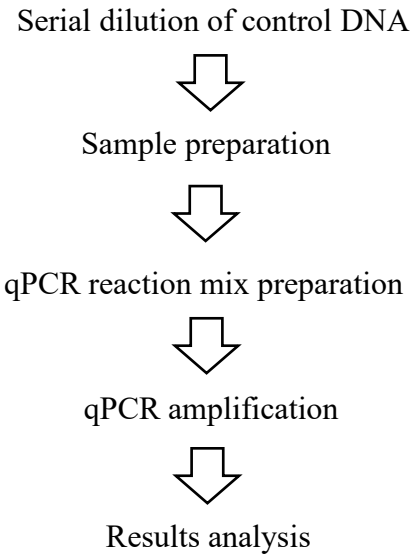
■ Required materials not included in the kit

- 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 equipment

- Benchtop microcentrifuge
- Real-Time PCR system
- Vortex mixer
- Microplate shaker
- Micropipettes: 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, 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.

■ Human DNA Control serial dilutions for the standard curve

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

Please check the concentration on the label of the Human DNA Control prior to dilution.

Prepare Human DNA Control solution with DNA Dilution Buffer (DDB) following the serial dilution procedure below:

1. Thaw Human 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, respectively.

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 it thoroughly.

4. Add 180 μL DDB to each tube of ST1, ST2, ST3, ST4 and ST5.

5. Perform the serial dilutions according to Table 2:

Table 2. Dilution for Human 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*

■ Sample preparation

➢ Negative Control Sample (NCS) Preparation

Add 100 μL DDB into a 1.5 mL clean centrifuge tube and label as NCS.

NCS and samples are prepared in the same experiment 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 = (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:

Table 3. qPCR MIX-75 Preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
Human Primer&Probe MIX-75	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 4. qPCR MIX-122 Preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
Human Primer&Probe MIX-122	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 5. qPCR MIX-244 Preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
Human Primer&Probe MIX-244	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 6. qPCR MIX-562 Preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
Human Primer&Probe MIX-562	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.

■ qPCR Reaction MIX preparation

1. Prepare qPCR Reaction MIX following Table 7-10, and a 96-well plate layout template is shown in Table 11.

Table 7. qPCR Reaction MIX-75 Preparation

Tubes	qPCR MIX-75	Samples	Total Volume
ST-75	20 μL	10 μL ST1/ST2/ST3/ST4/ ST5	30 μL
NTC	20 μL	10 μL DDB	30 μL
NCS	20 μL	10 μL purified NCS	30 μL
Test sample	20 μL	10 μL purified test sample	30 μL

Table 8. qPCR Reaction MIX-122 Preparation

Tubes	qPCR MIX-122	Samples	Total Volume
ST-122	20 μL	10 μL ST1/ST2/ST3/ST4/ ST5	30 μL
NTC	20 μL	10 μL DDB	30 μL
NCS	20 μL	10 μL purified NCS	30 μL
Test sample	20 μL	10 μL purified test sample	30 μL

Table 9. qPCR Reaction MIX-244 Preparation

Tubes	qPCR MIX-244	Samples	Total Volume
ST-244	20 μL	10 μL ST1/ST2/ST3/ST4/ ST5	30 μL
NTC	20 μL	10 μL DDB	30 μL
NCS	20 μL	10 μL purified NCS	30 μL
Test sample	20 μL	10 μL purified test sample	30 μL

Table 10. qPCR Reaction MIX-562 Preparation

Tubes	qPCR MIX-562	Samples	Total Volume
ST-562	20 μL	10 μL ST1/ST2/ST3/ST4/ ST5	30 μL
NTC	20 μL	10 μL DDB	30 μL
NCS	20 μL	10 μL purified NCS	30 μL
Test sample	20 μL	10 μL purified test sample	30 μL

Table 11. Example of 96-well plate layout

MIX-75			MIX-122			MIX-244			MIX-562			
NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	NTC	A
NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	NCS	B
S	S	S	S	S	S	S	S	S	S	S	S	C
ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	ST5	D
ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	ST4	E
ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	ST3	F
ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	ST2	G
ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	ST1	H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents four assays, selected standard curve points of Human DNA Control (ST1-ST5),1 NTC,1 NCS and 1 test sample, and 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 11.*

2. Seal the 96-well plate with sealing film. Mix it well in a microplate shaker, then spin down the reagents for 10 seconds in centrifuge and place it onto the qPCR instrument.

■ qPCR program setting

Note:The following instructions apply only to the 7500 instrument(passive reference dye ROX included) 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 Human-75 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 Human-122, Human-244 and Human-562, separately as step 2.

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 qPCR program as following:

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 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: target DNA detector task= **Unknown**

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

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 of Human-75, enter 0.04;
   c. In the Threshold field of Human-122, Human-244 and Human-562, enter 0.02.
   d. 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. Set the value of 75 fragment to be 100%, calculate the percentage of the 122, 244 and 562 fragments.
11. The Ct value of IPC need to be anaylzed. In princeple, the mean Ct-IPC value of the sample should be within ±1.0 of the NCS. If the mean Ct-IPC value of the sample is significantly higher than the NCS, this indicates that the sample may be inhibitory to the assay. If you have included ERC sample in parallel, then consider sample recovery rate prior to IPC results, and IPC results can only be used as reference.

12. The Ct value of NTC and NCS should be larger than the mean Ct value of the lowest standard curve concentration in FAM channel. And it shows normal amplification curve of a typical “S” shape in VIC channel.

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