SHENTEK

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

Version: A/1
For Research Use Only
Product No.: 1103170-2
Reagents for 4×100 Reactions

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

1. Product information

■ Product description

SHENTEK® Residual CHO DNA Size Analysis Kit (2G) is used to quantitate CHO DNA residues of different fragment sizes in different stages of biopharmaceutical products, from in-process samples to final products. This kit utilizes fluorescent quantitative PCR technique (FAM) to perform rapid and specific quantitation of residual CHO DNA fragments in samples. The assay is designed to amplify four different fragments (95bp, 110bp, 215bp, 523bp) for the accurate size distribution, and the detection limit 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
qPCR Reaction Buffer	NNB002	850 µL × 8 tubes	-20℃, protect from light
CHO Primer&Probe MIX-95	NNC087	300 µL × 1 tube	
CHO Primer&Probe MIX-110	NNC088	300 µL × 1 tube	
CHO Primer&Probe MIX-215	NNC089	300 µL × 1 tube	
CHO Primer&Probe MIX-523	NNC090	300 µL × 1 tube	
IPC MIX	NNC069	550 µL × 1 tube	
CHO DNA Control	NNA001	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
- Linegene 9600 plus Real-Time PCR System

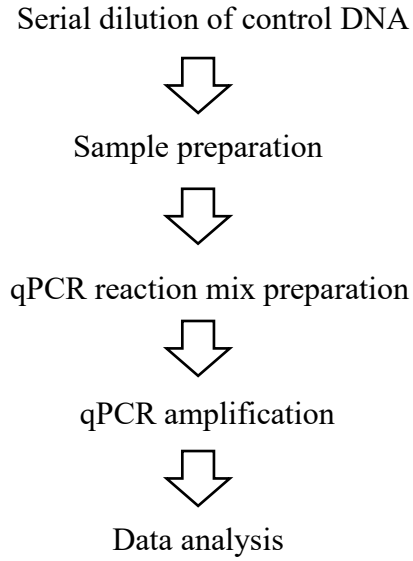
■ Required materials not included in the kit

- Nonstick, DNase-free & Low Retention Microfuge Tubes of 1.5 or 2.0 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

■ Related equipments

- Benchtop microcentrifuge
- Vortex mixer
- Micropipettes of 1000 µL, 100 µL and 10 µL
- Real-time PCR system
- Microplate 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% alcohol.

3. Thaw the kit completely at 2-8°C or melt on ice.

■ DNA Control serial dilutions for the standard curve

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

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

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

1. Thaw CHO 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 the serial dilutions according to Table 2:

Table 2. Dilution for CHO 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

➢ Test Sample Preparation

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

➢ Extraction Reference Control (ERC) samples Preparation

According to the CHO DNA spike concentration in ERC samples (Take the samples containing 30 pg of CHO DNA as example), the specific preparation procedure is as follows:

(1) Take 100 μL of the test sample and add it to a new 1.5 mL microfuge tube.

(2) Add another 10 μL of ST3, mix thoroughly and lable it as the ERC sample.

➢ 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 seperately according to Table 3 - 6.

Table 3. qPCR MIX-95 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
CHO Primer&Probe MIX-95	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 4. qPCR MIX-110 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
CHO Primer&Probe MIX-110	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 5. qPCR MIX-215 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
CHO Primer&Probe MIX-215	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 μL
Total volume	20 μL	660 μL

Table 6. qPCR MIX-523 preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
qPCR Reaction Buffer	15.9 μL	524.7 μL
CHO Primer&Probe MIX-523	2.8 μL	92.4 μL
IPC MIX	1.3 μL	42.9 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 Reactions MIX according to Table 7 - 10, and a 96-well plate layout template is shown in Table 11.

Table 7. qPCR Reaction MIX-95 preparation

Tubes	Standard curver-95	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-110 preparation

Tubes	Standard curver-110	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-215 preparation

Tubes	Standard curver-215	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-523 Preparation

Tubes	Standard curver-523	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-95						MIX-110					
A	ST1	ST1	ST1	S1	S2	NTC	ST1	ST1	ST1	S1	S2	NTC
B	ST2	ST2	ST2	S1	S2	NTC	ST2	ST2	ST2	S1	S2	NTC
C	ST3	ST3	ST3	S1	S2	NTC	ST3	ST3	ST3	S1	S2	NTC
D	ST4	ST4	ST4				ST4	ST4	ST4			
E	ST5	ST5	ST5	S1 ERC	S2 ERC	NCS	ST5	ST5	ST5	S1 ERC	S2 ERC	NCS
F				S1 ERC	S2 ERC	NCS				S1 ERC	S2 ERC	NCS
G				S1 ERC	S2 ERC	NCS				S1 ERC	S2 ERC	NCS
H												
	1	2	3	4	5	6	7	8	9	10	11	12

Plate 2：

	MIX-215						MIX-523					
A	ST1	ST1	ST1	S1	S2	NTC	ST1	ST1	ST1	S1	S2	NTC
B	ST2	ST2	ST2	S1	S2	NTC	ST2	ST2	ST2	S1	S2	NTC
C	ST3	ST3	ST3	S1	S2	NTC	ST3	ST3	ST3	S1	S2	NTC
D	ST4	ST4	ST4				ST4	ST4	ST4			
E	ST5	ST5	ST5	S1 ERC	S2 ERC	NCS	ST5	ST5	ST5	S1 ERC	S2 ERC	NCS
F				S1 ERC	S2 ERC	NCS				S1 ERC	S2 ERC	NCS
G				S1 ERC	S2 ERC	NCS				S1 ERC	S2 ERC	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 CHO DNA Control (ST1～ST5), 1 NTC, 1 NCS, 2 test samples(S1-S2) and 2 ERC samples (S1 ERC to S2 ERC), 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 it 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. Click **New Detector**, then enter CHO-95 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 CHO-110, CHO-215 and CHO-523, 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 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. Set the value of DNA size-95 to be 100%, calculate the percentage of the DNA size of 110, 215 and 523.

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.

Effective date: 09 Jul. 2024

Support & Contact

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