NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

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SHENTEK

Residual CHO DNA Quantitation Kit

User Guide

Version: A/1
For Research Use Only
Product No.: SK030201C100
Reagents for 100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual CHO DNA Quantitation Kit is used for quantitation of residual CHO host cell DNA in a variety of biopharmaceutical products. This kit uses quantitative PCR to perform rapid and specific quantitation of residual DNA at the femtogram level. For extraction information, please refer to the SHENTEK® Residual Host Cell DNA Sample Preparation Kit User Guide (Product No. SK030203D100).

■ 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
CHO DNA Control	NNA001	50 μL × 1 tube	-20℃
CHO qPCR MIX	NNC001	1 mL × 2 tubes	-20℃, protect from light
DNA Dilution Buffer (DDB)	NND001	1.5 mL × 3 tubes	-20℃

Note: According to USP <659>, items with recommended storage temperatures not exceeding –20°C shall be stored within ± 10°C (–30°C to –10°C). Short-term storage below –30°C is acceptable when supported by stability data.

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
- Mx3000P™ Real-Time PCR System
- qTOWER^3 G Real-Time PCR Thermal Cycler
- StepOnePlus™ 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
- Vortex mixer
- Benchtop microcentrifuge
- Pipettes: 1000 μL, 100 μL and 10 μL
- Microplate and microtube shaker

■ Workflow

Serial dilutions of the control DNA preparation

⇩

Sample preparation

⇩

qPCR reaction mix preparation

⇩

qPCR amplification

⇩

Results analysis

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 centrifuge briefly.

■ CHO DNA Control serial dilutions for the standard curve

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

Prepare a series of CHO DNA Control solution with DNA Dilution Buffer (DDB) as follows:

1. Thaw CHO DNA Control and DNA Dilution Buffer completely at 2-8°C or melt on ice. Vortex to mix well and centrifuge briefly, and repeat 3 times.
2. Label seven nonstick 1.5 mL centrifuge tubes as ST0, ST1, ST2, ST3, ST4, ST5 and ST6, respectively.
3. Transfer a certain amount of DNA Dilution Buffer and CHO DNA Control to ST0 tube to achieve a 3000 pg/μL control solution.

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

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 calculated volume below to reach 3000 pg/μL.

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

4. Vortex to mix well and centrifuge briefly the ST0 tube for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.
5. Add 90 μL DDB to all tubes of ST1, ST2, ST3, ST4, ST5 and ST6.
6. 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	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
ST6	10 μL ST5 + 90 μL DDB	0.003

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

■ Sample preparation

➢ Preparation of Extraction Reference Control (ERC) samples

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. Aliquot 100 μL of the test sample to a new 1.5 mL centrifuge tube.

2. Add 10 μL of ST3 solution and mix thoroughly, label as the ERC sample.

➢ Preparation of Negative Control Samples (NCS)

Add 100 μL of DDB to a new 1.5 mL centrifuge tube, and label as Negative Control Sample (NCS).

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

■ qPCR Reaction MIX preparation

1. After thoroughly mixing CHO qPCR MIX, follow 20 µL each tube is divided into PCR 8-well strip tubes or 96-well plate.

2. Prepare qPCR Reaction MIX according to Table 3 and 96-well plate layout as shown in Table 4.

Table 3. qPCR Reaction MIX preparation

Tubes	Standard curve	NTC	NCS	Test sample
qPCR MIX	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 4. Example of 96-well plate layout

NTC		S1	S1	S1	S1 ERC	S1 ERC	S1 ERC		ST6	ST6	ST6	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 6 concentration gradients (ST1 to ST6), 1 NTC, 1 NCS, and 5 test samples (S1 to S5), and 5 ERC samples (S1 ERC to S5 ERC), and 3 replicates for each sample.*
- *In specific testing, the plate layout for sample loading can be adjusted based on the sample quantity.*

3. Seal the 96-well plate with sealing film. Mix it well in microplate shaker, then spin down the reagents for 10 seconds in centrifuge and place it in 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**, then enter CHO-DNA in the Name field.
4. Select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
5. Add CHO-DNA to **Detectors in Document**.
6. Select **ROX** as the passive reference dye, then click **Next**.
7. Select the applicable set of wells for the samples, then select CHO-DNA detector for each well.
8. Select **Finish**, and then set thermal-cycling conditions:
   a. Choose the thermal cycling reaction volume to 30 μL.
   b. Set up the program as following:

Table 5. 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.

9. 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 the following table:

Table 6. Settings for Standard curve

Tube label	Task	Conc. (pg/μL)
ST1	Standard	300
ST2	Standard	30
ST3	Standard	3
ST4	Standard	0.3
ST5	Standard	0.03
ST6	Standard	0.003

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. The recovery of ERC samples is calculated based on the results of the test samples and the ERC samples. The recovery should be between 50% and

150%.

11. The Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve, and if the proven quantitation limit (QL) is lower than the minimum standard curve concentration, the detected value of NCS should be less than the quantitation limit (QL).
12. The Ct value of NTC should be Undetermined or not less than 35.00, or set specific standards based on the laboratory's own validation results.

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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Support & Contact

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