SHENTEK

Residual PG13 DNA Quantitation Kit
User Guide

Version: A/0
For Research Use Only
Product No.: 1101123
Reagents for 100 Reactions

Huzhou Shenke Biotechnology Co., Ltd

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

1. Product information

■ Product description

SHENTEK® Residual PG13 DNA Quantitation Kit is used to quantitate residual PG13 host cell DNA in different stages of biopharmaceutical products, from in-process samples to final products. This kit utilizes quantitative PCR (qPCR) technique to perform a rapid, specific, and reliable quantitation assay at the femtogram (fg) level. The kit provides PG13 DNA Control as reference standard. For extraction information, please refer to the SHENTEK® Residual Host Cell DNA Sample Preparation Kit User Guide.

■ 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
PG13 DNA Control	NNA049	50 μL × 1 tube	-20℃
qPCR Reaction Buffer	NNB001	850 μL × 2 tubes	-20℃, protect from light
PG13 Primer&Probe MIX	NNC101	300 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 480 II Real-Time PCR System

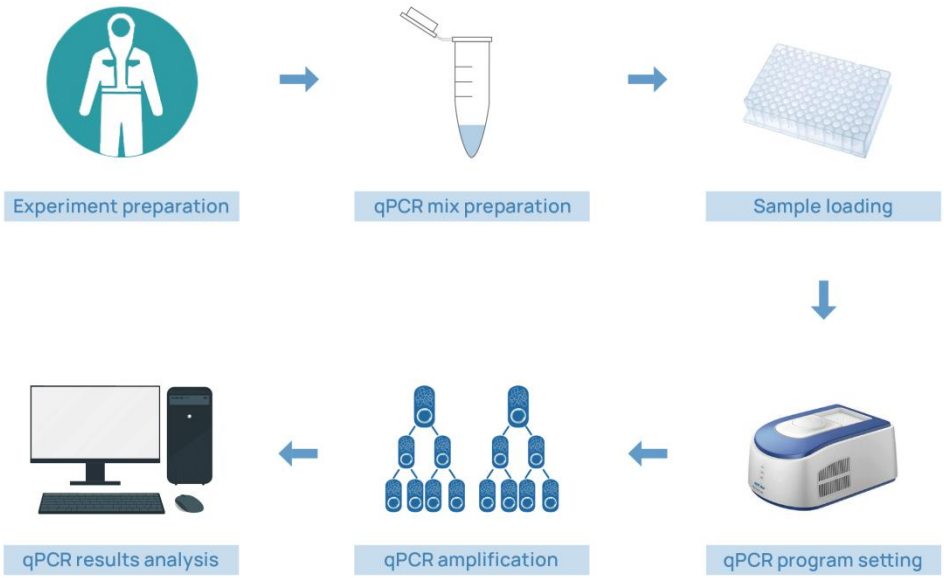
■ Required materials not included in the kit

- 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
- 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.

■ PG13 DNA Control serial dilutions for the standard curve

Please check the concentration labeled on the tube containing the PG13 DNA Control prior to dilution.

1. Thaw PG13 DNA Control and DNA Dilution Buffer (DDB) completely at 2-8°C or melt on ice. Vortex to mix well and briefly spin down the reagents for 3-5 seconds in a microcentrifuge, and repeat 3 times.
2. Label seven 1.5 mL microcentrifuge tubes: ST0, ST1, ST2, ST3, ST4, ST5 and ST6.
3. Dilute the PG13 DNA Control to 3000 pg/μL with DDB in the ST0 tube. Calculate the volume of DDB to prepare the ST0:

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

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 briefly spin down the ST0 tube for 3-5 seconds in microcentrifuge, and repeat 3 times to mix thoroughly.
5. Add 90 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 of PG13 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 needs to be stored at 2-8℃. If the solution is cloudy or contains precipitates, heat at 37℃ until it is 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

➢ Extraction Reference Control (ERC) Sample Preparation

According to the PG13 DNA spike concentration in ERC samples (take the sample containing 30 pg of PG13 DNA as example), specific preparation procedure is as follows:

(1) Take 100 μL of the test sample to a new 1.5 mL microcentrifuge tube.

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

➢ Positive Control Sample (PCS) Preparation

According to the PG13 DNA spike concentration in PCS (take the sample containing 30 pg of PG13 DNA as example), specific preparation procedure is as follows:

(1) Take 100 μL of DDB to a new 1.5 mL microcentrifuge tube.

(2) Add 10 μL of ST3 solution and mix thoroughly, label as PCS.

➢ Negative Control Sample (NCS) Preparation

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

- *ERC, PCS and 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 + 1 PCS + test samples)×3

2. Prepare qPCR MIX according to the number of reaction wells in Table 3.

Table 3. qPCR MIX preparation

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
qPCR Reaction Buffer	17 μL	561 μL
PG13 Primer&Probe MIX	3 μL	99 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-well strip tubes.

■ qPCR Reaction MIX preparation

1. Prepare qPCR Reaction MIX according to Table 4, and 96-well plate layout is shown in Table 5.

Table 4. qPCR Reaction MIX preparation

Tubes	Volume/reaction
Standard curve	20 μL qPCR MIX + 10 μL ST1/ST2/ST3/ST4/ ST5/ ST6
NTC	20 μL qPCR MIX + 10 μL DDB
NCS	20 μL qPCR MIX + 10 μL Extracted NCS
Test sample	20 μL qPCR MIX + 10 μL Extracted test sample
ERC sample	20 μL qPCR MIX + 10 μL Extracted ERC sample
PCS	20 μL qPCR MIX + 10 μL Extracted PCS

Table 5. Example of 96-well Plate layout

ST6	ST6	ST6		S1	S1	S1	S1 ERC	S1 ERC	S1 ERC		NCS	A
ST5	ST5	ST5		S2	S2	S2	S2 ERC	S2 ERC	S2 ERC		NCS	B
ST4	ST4	ST4		S3	S3	S3	S3 ERC	S3 ERC	S3 ERC		NCS	C
ST3	ST3	ST3		S4	S4	S4	S4 ERC	S4 ERC	S4 ERC			D
ST2	ST2	ST2		S5	S5	S5	S5 ERC	S5 ERC	S5 ERC		NTC	E
ST1	ST1	ST1									NTC	F
											NTC	G
				PCS	PCS	PCS						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-ST6), 1 NTC, 1 NCS, 1PCS, 5 test samples (S1-S5), and 5 ERC samples (S1 ERC-S5 ERC), with triplicates 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 5.*

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

■ qPCR program setting

NOTE: The following instructions apply only to the ABI 7500 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:**
   a. Enter PG13-DNA in the Name field.
   b. Select **FAM** in the Reporter Dye drop-down list and select **(none)** in the Quencher Dye drop-down list, then click **OK**.
   c. Select a color for the detector, then click **Create Another**.
4. Select **ROX** as the passive reference dye, then click **Next**.
5. Select the applicable set of wells for the samples, then select PG13-DNA detector for each well.
6. 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 follow in Table 6:

Table 6. 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.

7. 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 sample, PCS, and ERC sample: target DNA detector task =**Unknown**
2. Set up the standard curve as shown in table 7:

Table 7. 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

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, PG13-DNA enter 0.02.
   c. Select **Auto Baseline**.
6. Click the button ▶ in the toolbar, then wait the plate analyzing.

7. Select the **Results** 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. In the Report panel of Results, the 'Mean Qty' column shows the detection values of NTC, NCS, test sample, PCS, and ERC sample, in pg/μL.

Note: The parameter settings of the result analysis should be configured on the specific model and the software version, and generally can also be automatically interpreted by the instrument.

11. The recovery of ERC samples is calculated based on the value of the test samples and the ERC samples. The recovery should be between 50% and 150%.
12. The Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve. If the validated quantitation limit (QL) concentration is less than the lowest concentration of the standard curve, the value of the NCS should be less than the concentration of QL.
13. The Ct value of NTC should not be less than 35.00 cycles or undetermined, or set standards based on the laboratory validation results.

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Support & Contact

SHENTEK

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou 313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189