NEED HELP?

User Manual

Disclaimer: Products are intended for research use only

SHENTEK Biofargo

www.biofargo.com
804-529-2296
contact@biofargo.com

SHENTEK

Residual *Pichia Pastoris* DNA Quantitation Kit User Guide

Version: A/1
For Research Use Only
Product No.: SK030205P100
Reagents for 100 Reactions

Biofargo, Inc.

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

1. Product information

■ Product description

SHENTEK® Residual *Pichia Pastoris* DNA Quantitation Kit is used to quantitate residual *Pichia Pastoris* DNA 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 *Pichia Pastoris* DNA fragments in samples, and reliable quantitation assay at the fg level. *Pichia Pastoris* DNA Control is provided as reference standard. 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
Pichia Pastoris DNA Control	NNA007	50 μL × 1 tube	-20℃
Pichia Pastoris qPCR Reaction Buffer	NNB005	850 μL × 2 tubes	-20℃, protect from light
Pichia Pastoris Primer&Probe MIX	NNC041	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
- 7500 Real-Time PCR System
- Linegene 9600plus Real-Time PCR System
- CFX96 Real-Time PCR System

- StepOne Plus Real-Time PCR System

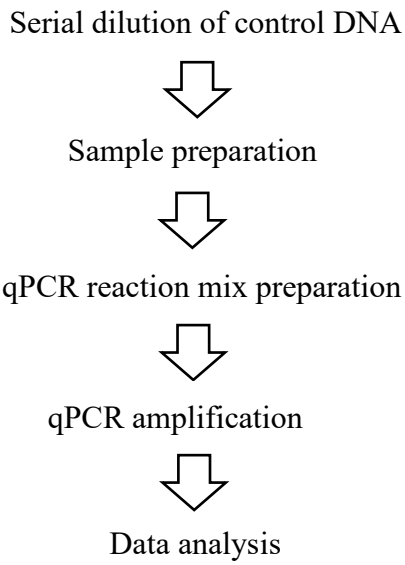
■ Required materials not included in the kit

- Nonstick, DNase-free, Low Retention Microfuge Tubes, 1.5mL
- 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 equipment

- Bentchtop microcentrifuge
- Vortex mixer
- Micropipettes of 1000 μL, 100 μL, 10 μL
- Real-time PCR instrument
- 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

Please check the concentration on the label of each tube containing the *Pichia Pastoris* DNA Control prior to dilution.

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

1. Thaw *Pichia Pastoris* 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 90 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 *Pichia Pastoris* DNA Control

Serial dilution tube	Dilution	Conc. (pg/μL)
ST0	Dilute the DNA Control	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

- *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 to a new 1.5 mL microfuge tube.

- Extraction Reference Control (ERC) samples Preparation

  According to the *Pichia Pastoris* DNA spike concentration in ERC samples (Take the samples containing 30 pg of *Pichia Pastoris* DNA as example), 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 microcentrifuge 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 the number of reaction wells.

Table 3. qPCR MIX preparation

Reagents	**Volume/reaction**	**Volume for 30 reaction (includes 10% overage)**
Pichia Pastoris qPCR Reaction Buffer	17 μL	561 μL
Pichia Pastoris 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-strip tubes.

■ qPCR Reaction mix preparation

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

Table 4. 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 - ST5	10 μL DDB	10 μL purified NCS	10 μL purified test sample
Total Volume	30 μL	30 μL	30 μL	30 μL

Table 5. Example of 96-well plate layout

NTC		S1	S1	S1	S1 ERC	S1 ERC	S1 ERC		ST5	ST5	ST5	A
NTC		S2	S2	S2	S2 ERC	S2 ERC	S2 ERC		ST4	ST4	ST4	B
NTC		S3	S3	S3	S3 ERC	S3 ERC	S3 ERC		ST3	ST3	ST3	C
		S4	S4	S4	S4 ERC	S4 ERC	S4 ERC		ST2	ST2	ST2	D
NCS		S5	S5	S5	S5 ERC	S5 ERC	S5 ERC		ST1	ST1	ST1	E
NCS												F
NCS												G
												H
1	2	3	4	5	6	7	8	9	10	11	12	

- *This example represents four assays, including selected standard curve points of Pichia Pastoris DNA Control (ST1-ST5), 1 NTC, 1 NCS, 5 ERC samples (S1 ERC to S5 ERC) and 5 test sample(S1-S5), 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 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 *Pichia Pastoris* DNA 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. Select **ROX** as the passive reference dye, then Click **Next**.
4. Select the applicable set of wells for the samples, then select the corresponding detector for each well.
5. 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 following (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℃*	01 :00	

* Instrument will read the fluorescence signal during this step.

6. 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 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

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. In the Report panel of Results, the 'Mean Quantity' column shows the detection values of NTC, NCS, ERC, test sample, in pg/μL.
11. Calculate the spiked recoveries from the results of the tested samples and the sample ERC, and it is required to be in the range of 50%-150%.
12. The Ct value of NCS should be larger than the mean Ct value of the lowest concentration in the standard curve.
13. The Ct value of NTC should be no less than 35.00 cycles, or specific standards can be set based on the results of internal laboratory validation.

    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

Biofargo, Inc.
www.biofargo.com
Address: 1716 E Parham Rd Richmond, Va, 23228, USA
E-mail: contact@biofargo.com
Phone: 804-529-2296