

KRISHGEN® Residual Pichia Pastoris DNA Quantitation RT-PCR Kit



Instruction Manual



KBBR7212



Store the kit and its components at -20°C

For Research Use Only

RUO

100 reactions

Introduction:

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 Residual Host Cell DNA Sample Preparation Kit.

Intended Use:

The KRISHGEN® Residual Pichia Pastoris DNA Quantitation RT-PCR Kit can be used to quantitate Pichia Pastoris DNA in different stages of biopharmaceutical products, from in-process samples to final products.

Kit Components:

1. Pichia Pastoris DNA Control (concentrated, indicated on vial label) – 50 ul x 1 vial
2. Pichia Pastoris DNA qPCR Reaction Buffer – 850 ul x 2 vials
3. Pichia Pastoris DNA Primer & Probe Mix – 300 ul x 1 vial
4. DNA Dilution Buffer (DDB) – 1.5 ml x 3 vials
5. Instruction Manual

Additional Requirements to be provided by the End-user:

1. DNase-free, Low Retention Microfuge Tubes, 1.5ml
2. Low Retention Tips of 1000 ul, 100 ul, 10 ul.
3. 96-well qPCR plates with sealing film or PCR 8-strip tubes with caps
4. Microcentrifuge
5. Vortex mixer
6. Micropipettes of 1000 ul, 100 ul, 10 ul
7. Real-time PCR instrument
8. Microplate shaker

Applied instruments, including but not limited to the following:

1. ABI 7500 Real-Time PCR System
2. CFX96 Real-Time PCR System
3. 96S Real-Time PCR System
4. Lightcycler 480 Real-Time PCR System

Stability and Storage:

Store the DNA Control, qPCR Reaction Buffer, Primer and Probe MIX at -20°C, protect from light.

General Precautions

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.

- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
Avoid samples and reagents contact with the skin, eyes, and mucous membranes. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice immediately.
- Safety Data Sheets (SDS) is available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.
Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

Reagent Preparation:

DNA Standard serial dilutions for the standard curve

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

- Thaw Pichia Pastoris DNA Control and DNA Dilution Buffer 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. Repeat this 3 times.
- Label six non-stick 1.5 ml centrifuge tubes: ST0, ST1, ST2, ST3, ST4 and ST5.
- Transfer a certain amount of DNA Dilution Buffer and Pichia Pastoris DNA Control to ST0 tube to achieve a 3000 pg/ul control solution. Vortex to mix well and quickly spin down and repeat 3 times.
- Add 90 ul DNA Dilution Buffer to all tubes of ST1, ST2, ST3, ST4 and ST5.
- Perform the serial dilutions according to below table.

Standard Concentration (pg/ul)	Standard Vial	Dilution Particulars
0.03	ST5	10 ul Standard No.4 + 90 ul DNA Dilution Buffer
0.3	ST4	10 ul Standard No.3 + 90 ul DNA Dilution Buffer
3	ST3	10 ul Standard No.2 + 90 ul DNA Dilution Buffer
30	ST2	10 ul Standard No.1 + 90 ul DNA Dilution Buffer
300	ST1	10 ul Standard No.0 + 90 ul DNA Dilution Buffer
3000	ST0	Dilute the DNA Control with DDB

Note:

- The remaining unused DNA Dilution Buffer need to be stored at 2-8°C. If the solution is cloudy or contains precipitates, heat at 37°C until it is 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 ul of the test sample and add to a 1.5 ml micro centrifuge tube.

Extraction Reference Control (ERC) Sample Preparation

According to the Pichia Pastoris DNA spike concentration in ERC samples (Take the samples containing 30 µg 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 solution and mix thoroughly, label as ERC sample.

Negative Control Sample (NCS) Preparation

Add 100 µl of DNA Dilution Buffer to a new 1.5 ml micro centrifuge tube, and label it as NCS.

Note: NCS and samples should be prepared in same way for DNA extraction.

Assay Protocol

A) 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 as mentioned below:

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 the above prepared qPCR Mix and place it on ice, aliquot 20 µL/well into 96-well qPCR plate or PCR 8-strip tubes.

B) qPCR Reaction mix preparation:

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

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

Chart: Reference 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 the assay for a standard curve with 5 concentration gradients (ST1-ST5), 1 NTC, 1 NCS, 5 test samples (S1 to S5), and 5 ERC samples (S1 ERC to S5 ERC), with 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 chart.

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.

C) qPCR Instrument Running Program Settings:

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, and then in the Assay drop-down list, select Standard Curve (Absolute Quantitation).
2. Click New Detector:
 - a. Enter Pichia Pastoris 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, and then click **OK**.
3. Select **ROX** as the passive reference dye, and then click **next**.
4. Select the applicable set of wells for the samples, and 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 ul.
 - b. Set the temperature and time as following:

qPCR Running Temperature and Time:

Step	Temp.	Time (mm:sec)	Cycles
Activation	95°C	10:00	1
Denaturation	95°C	00:15	40
Annealing/extension	60°C*	01:00	

*Instrument will read the fluorescence signal during this step.

8. Save the document, then click **Start** to start the real time qPCR run.

NOTE: The following instructions apply only to the ABI7500 instrument. If you use a different instrument or software, please contact us at sales1@krishgen.com.

Calculation of Results:

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:

Settings for Standard curve

Tube label	Task	Quantity (pg/ul)
ST1	Standard	300
ST2	Standard	30
ST3	Standard	3
ST4	Standard	0.3
ST5	Standard	0.03

3. Select the **Results** tab, and 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, and then wait the plate analyzing.
7. Select the **Result** tab >> **Standard curve** tab, then verify the Slope, Intercept and R² values.
8. Select the Report tab, and 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 can read the detection values of NTC, NCS, ERC, test sample, in pg/ul.
11. The recovery rate of ERC samples is calculated based on the value of test samples and the ERC samples. The recovery rates 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 and it shows normal amplification curve in the VIC signal channel.
13. The detection value of NTC should be no less than 35.00 cycles, or set specific standards based on the laboratory's own validation results.

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.

Symbols



Use by



Lot/Batch



Catalog number



Temperature limitation



Caution, consult accompanying documents



Manufacturer

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems Private Limited. 2026

THANK YOU FOR USING A KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED®, DHARMAPLEX®, GENBULK®, GENLISA®, KRISHZYME®, KRISHGEN®, KRISHPLEX®, TITANIUM®, QUALICHEK® are registered trademarks of KRISHGEN BIOSYSTEMS PRIVATE LIMITED.

©KRISHGEN BIOSYSTEMS PRIVATE LIMITED. ALL RIGHTS RESERVED.

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |