KRISHGEN® Residual CHO DNA Quantitation RT-PCR Kit

Instruction Manual

REF **KBBR7121**

Store the kit and its components at -20ºC

For Research Use Only **RUO**

100 reactions

Introduction:

Residual CHO DNA Quantitation Kit is used to quantitate host-cell line residual DNA from CHO cell line, which is used for production of biopharmaceutical products. This kit uses duplex real-time PCR technology to perform rapid, specific, and reliable quantitation assay at the fg level. IPC (Internal Positive Control) is included in the CHO Primer & Probe MIX to evaluate the performance of each PCR reaction.

Intended Use:

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

Kit Components:

1. Residual CHO DNA Control – 50 ul x 1 vial
2. Residual CHO qPCR Master Mix – 850 ul x 2 vials
3. DNA Dilution Buffer (DDB) – 1.5 ml x 3 vials
4. Residual CHO Primer & Probe Mix – 500 ul x 1 vial
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. StepOne Plus Real-Time PCR System
4. Real Time PCR System, compatible

Stability and Storage:

Store the Residual CHO DNA Control, qPCR Reaction Buffer, Primer and Probe MIX at -20ºC, 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

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

a. Thaw Residual CHO DNA Control and DNA Dilution Buffer completely at 2-8ºC or melt on ice. Vortex to mix well and quickly spin down the reagents for 3-5 seconds in micro centrifuge. Repeat this 3 times.
b. Label seven non-stick 1.5 ml microfuge tubes: ST0, ST1, ST2, ST3, ST4, ST5 and ST6.
c. Dilute the CHO DNA Control to 3000 pg/ul 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 it thoroughly.
d. Add 90 ul Dilution Buffer to each tube: ST1, ST2, ST3, ST4, ST5, ST6.
e. Perform the serial dilutions according to below table
f.

Standard Concentration Dilution	Standard Vial	Dilution Particulars
3000 pg/ul	ST0	Dilute the DNA Control with DDB
300 pg/ul	ST1	10 ul ST0 + 90 ul DDB
30 pg/ul	ST2	10 ul ST1 + 90 ul DDB
3 pg/ul	ST3	10 ul ST2 + 90 ul DDB
0.3 pg/ul	ST4	10 ul ST3 + 90 ul DDB
0.03 pg/ul	ST5	10 ul ST4 + 90 ul DDB
0.003 pg/ul	ST6	10 ul ST5 + 90 ul DDB

Note:

- *The remaining unused DNA Dilution Buffer need to be stored at 2-8℃. If the solution is cloudy or contains precipitates, heat at 37℃ 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

Spike the Residual CHO DNA in ERC samples.
Take the samples containing 30 pg of Residual CHO DNA.
Add 100 ul of the test sample and add it to a new 1.5 mL microfuge tube.
Add another 10 ul of ST3, mix thoroughly and label it as the ERC sample.

Negative Control Sample (NCS) Preparation
Add 100 ul of DDB 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.
2. Prepare qPCR Mix according to the number of reaction wells as mentioned below:

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
Residual CHO qPCR Reaction Buffer	15 ul	495 ul
Residual CHO Primer & Probe MIX (Incl IPC)	5 ul	165 ul
Total volume	20 ul	660 ul

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

B) qPCR Reaction mix preparation:

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	Test Sample ER
qPCR Mix	20 ul	20 ul	20 ul	20 ul	20 ul
Samples	10 ul ST1 – ST6	10 ul DDB	10 ul purified NCS	10 ul purified test sample	10 ul purified ERC sample
Total Volume	30 ul	30 ul	30 ul	30 ul	30 ul

Chart: Reference 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-ST6), 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. In the Run Mode drop-down list, select **Standard 7500**, and then click **next**.
3. Click New Detector:
   a. Enter Residual CHO 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.**
   c. Select a color for the detector, then click **Create Another.**
4. Click New Detector:
   a. Enter IPC in the name field.
   b. Select **VIC** in the reporter Dye drop-down list and select (**none**) in the Quencher Dye drop-down list, and then click **OK.**
   c. Select a color for the detector, then click **OK.**
   d. Select the detectors, then click Add to add the detectors to the document.
5. Select **ROX** as the passive reference dye, and then click **next.**
6. Select the applicable set of wells for the samples, and then select the Residual CHO DNA detector for each well.
7. 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:

Create a temperature profile on the RT PCR instrument as follows:

Step	Temperature	Time	Cycles
Activation	95°C	10 min	1
Denaturation	95°C	15 s	40
Annealing/Extension	60°C	1 min	

*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, and ERC wells: target DNA detector task = **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
ST6	Standard	0.003

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, CHO DNA enter 0.05 and IPC enter 0.1.
   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 R^2 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, test sample, and sample ERC, in pg/reaction.

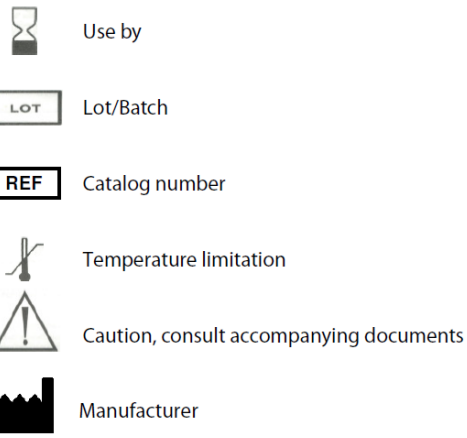
11. The recovery rate of ERC samples is calculated based on the value of test samples and the ERC samples. The recovery rates should be between 50% and 150%.

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 Ct value of NTC should be ≥ 35.00, or set specific standards based on the laboratory's own validation results, and it shows normal amplification curve in the VIC signal channel.

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



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 | OUR REAGENTS | YOUR RESEARCH |