EZassay

CHO Residual DNA qPCR Detection Kit

info@ezassay.com

www.ezassay.com

EZassay Biotechnology Ltd.

Catalog Code: CHO-DNA-PCR-100

CONTENTS

Contents	Page
Product Introduction	1
Materials supplied	1
Storage	1
Other materials required	1
Assay procedure	2

Product Introduction

Based on proven real-time qPCR technology, this kit is used to quantitate host‐cell residual DNA from Chinese hamster ovary (CHO) cells, a widely used cell line for production of biopharmaceutical products. Use the kit after you extract host‐cell DNA from test samples. For extraction information, refer to Residual DNA Sample Preparation Kit (magnetic bead based) (Cat. #:MB-WG-100)
This kit performs rapid, specific quantitation of sub‐picogram levels of residual CHO host‐cell DNA. **Linearity is demonstrated by analysis of CHO cell standard DNA ranging from 3 fg/µL to 300 pg/µL**, which meets the sensitivity requirements defined by WHO (10 ng CHO DNA per therapeutic dose).

Materials supplied

Component	Catalog number:： CHO-DNA-PCR-100 Volume(Package size： 100 tests.)
CHO qPCR Mix_Lyophilized*1 CHO Primer & Probe Mix_Lyophilized*2 CHO DNA Standard_Lyophilized*1	Add PCR-grade water according to the label on the tube before use. Note: Before opening the tube, briefly centrifuge at 3000–4000 rpm for 1 minute to collect the reagents at the bottom of the tube.
DNA Dilution Buffer	1500 µL *4
PCR-grade Water	1 vial

Storage

-20°C. Suggest aliquot. Avoid repeated freeze-thaw.

Other materials required

i. Q-PCR machine with FAM channel
Compatible with but not limited to following brand: Bio-Rad; CFX96 Optic Module；
Thermo Scientific： ABI 7500； ABI Quant Studio 5； ABI Step OnePlus； LineGene 9600.
ii. Centrifuge, vortex, pipette, laminar flow hood
iii. Sterile low-retention centrifuge tubes. (1.5 mL or 2.0 mL)
iv. Aerosol-resistant micropipette tips (1000 µL, 100 µL, 10 µL)
v. PCR tubes or PCR plates.
vi. PCR grade water.

Assay procedure

1. Preparation of the DNA Standard Dilutions for the Standard Curve

1.1. Thaw CHO DNA standard (30ng/µL) and Dilution buffer on ice or at 2-8°C. Gently flick and quick spin. Repeat 3 times to mix thoroughly.
1.2. Label ST0，ST1，ST2，ST3，ST4，ST5，ST6 on 1.5mL tubes.
1.3. To prepare ST0, dilute CHO DNA standard (30ng/µL) 1:10 with dilution buffer by adding 10µL CHO DNA standard to 90µL dilution buffer. (final concentration of DNA 3ng/µL) Gently flick and quick spin. Repeat 3 times to mix thoroughly.
1.4. Add 90µL Dilution buffer in each tube (ST1，ST2，ST3，ST4，ST5，ST6)
1.5. Further serial dilutions for ST1 to ST6 can be prepared starting with ST0, according to the Table 1.

Tube label	Dilution	Final concentration
ST1	10 µL ST0+90 µL DNA Dilution Buffer	300 pg/µL
ST2	10 µL ST1+90 µL DNA Dilution Buffer	30 pg/µL
ST3	10 µL ST2+90 µL DNA Dilution Buffer	3 pg/µL
ST4	10 µL ST3+90 µL DNA Dilution Buffer	300 fg/µL
ST5	10 µL ST4+90 µL DNA Dilution Buffer	30 fg/µL
ST6	10 µL ST5+90 µL DNA Dilution Buffer	3 fg/µL

Note:
The Standards ST0 to ST6 can be stored at 2~8°C for just the day of the preparation; otherwise they have to be stored at -15 to -25°C and used within one week.
When you prepare the serial dilutions of CHO DNA, follow these rules to avoid carryover contamination and to ensure proper sample preparation and quantitative PCR (qPCR) of samples:
- Use low-retention tubes.
- Label the top of each tube for identification.
- Use 2 sets of pipettes:

– For serial dilutions and to create the standard curve
– For sample preparation or to set up PCRs.
- Prepare the serial dilutions in an area physically separate from the test‑sample preparation area.

2. Preparation of Extraction Recovery Control (ERC)

Extraction/recovery control (ERC) can be used to assess the efficiency of DNA extraction, recovery, and quantitation from test samples. Additionally, you can use ERC to verify assay and system performance. The following procedure describes the preparation of an ERC sample containing 30 pg of CHO DNA control per well for qPCR analysis.

2.1. Transfer 100µL test sample to a clean tube. (1.5 mL)

2.2. Add 10 µL ST3, mix well, label as ERC.

2.3. When you finish, extract DNA from the tubes according to the Residual DNA Sample Preparation Kit, then quantify the extracted DNA in each tube as described in the following section

Note:

Adjust the amount of CHO DNA control added to the sample for those test samples that contain higher background levels of DNA. To assure accurate results, the amount of CHO DNA control that you add to a test sample should be approximately double the amount of DNA measured in the test sample without the addition of the CHO DNA control. To calculate the efficiency of DNA recovery and quantitation from the test samples, subtract the amount of DNA measured in the sample without the addition of CHO DNA control from the amount of DNA measured in the ERC sample.

3. Preparation of Negative Extraction Control (NEC)

Set up the negative extraction control as below.

3.1. Transfer 100µL sample solution into a clean 1.5 mL tube and label as NEC. For example, sample solution can be fresh medium.

3.2. Use NEC for sample preparation along with the same batch of samples to be tested. The NEC extraction is prepared.

4. Preparation of No Template Control (NTC)

4.1. Use PCR water or Dilution buffer as NTC.

5. Preparation of qPCR reaction master mix

5.1. According to the standard curve to be tested and the number of samples to be tested, calculate the number of reaction wells required, and generally make 3 replicate wells for each sample.
N=（6 Standards + 1 NTC+ 1 NEC +Test samples×2）×3;
（Test samples×2 is because ERC is recommended to be prepared for each sample）;
5.2. Thaw reagents on ice or 2~8 ℃. Prepare qPCR reactions as described in the table below.

Item	Volume for each well
CHO qPCR mix	15 µL
CHO Primer & probe mix	5 µL
Total volume	20 µL

5.3. Calculate the total volume of qPCR reaction required for this time based on the number of wells (with 2 wells lost)
Total volume of qPCR reaction = (number of wells+2) × 20 µL
5.4. Add samples as described in the table below.

Standard curve	20 µL CHO qPCR reaction master mix + 10 µL ST1/ST2/ST3/ST4/ST5/ST6
NTC	20 µL CHO qPCR reaction master mix + 10 µL DNA Dilution buffer
NEC	20 µL CHO qPCR reaction master mix + 10 µL NEC
Test samples	20 µL CHO qPCR reaction master mix + 10 µL Test samples
ERC	20 µL CHO qPCR reaction master mix + 10 µL ERC

Mix well and spin down.

6. Example of layout.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC		S1	S1	S1	S1 ERC	S1 ERC	S1 ERC		ST6	ST6	ST6
B	NTC		S2	S2	S2	S2 ERC	S2 ERC	S2 ERC		ST5	ST5	ST5
C	NTC		S3	S3	S3	S3 ERC	S3 ERC	S3 ERC		ST4	ST4	ST4
D			S4	S4	S4	S4 ERC	S4 ERC	S4 ERC		ST3	ST3	ST3
E	NEC		S5	S5	S5	S5 ERC	S5 ERC	S5 ERC		ST2	ST2	ST2
F	NEC									ST1	ST1	ST1
G	NEC											
H												

S=Sample；NTC=No Template Control； ERC=Extraction/Recovery Control；
NEC= Negative Extraction Control；ST1~ST6= standard

Note.

The plate layout is a suggested plate layout. Adjust the layout according to the number of test samples to be run.

7. Run the plate

7.1. Seal the plate with an optical film, then quick‐spin with a centrifuge rotor that is compatible with 96‐well plates. Load the prepared 96-well plate into the qPCR machine and start the run.

8. Create the plate document, run the plate, and analyze results

The following instructions apply only to the 7500 Fast instrument. If you use a different instrument, refer to the applicable instrument guide for setup guidelines

8.1. In the Template Assay drop‐down list, select Absolute Quantification.

8.2. In the Run Mode drop‐down list, select Standard 7500

8.3. Entery “CHO-DNA” in the Plate name field, then click Next.

8.4. Click New Detector:

a. Enter CHO in the Name field.

b. Select FAM in the Report Dye drop‐down list.

c. Select (none) in the Quencher Dye drop‐down list.

d. Select a color for the detector.

8.5. Select the Instrument tab, then set thermal‐cycling conditions:

- Set the thermal cycling reaction volume to 30 µL.
- For the 7500 Fast system, set the reaction to Standard.
- Set the temperature and the time as shown in the following table.

Step	Temperature	Time	Cycle	Note
1	95°C	5 minutes	1×	/
2	95°C 57°C	15 seconds 1 minute	40×	/ Read fluorescent signal

8.6. Save the document, then click Start to start the real‑time qPCR run.

9. Analyze the results

After the qPCR run is finished, use the following general procedure to analyze the results.

9.1. In the Analysis Settings window. Manually adjust the threshold line to the appropriate position. The appropriate position is just above the highest point of the amplification curve of the negative control. For example, enter 0.2 or 0.02 in the Threshold field. Click Analyze in the tool bar, then wait while the plate is analyzed.

9.2. Select the Results tab, Standard Curve tab.

Assign values of 3000, 300, 30, 3, 0.3, and 0.03 in the Quantity column. (meaning the total amount of DNA per well. The unit is pg.)

Name them ST1, ST2, ST3, ST4, ST5, and ST6 in the corresponding Sample Name column.

9.3. Set tasks for each sample type by clicking on the Task Column drop‑down list:

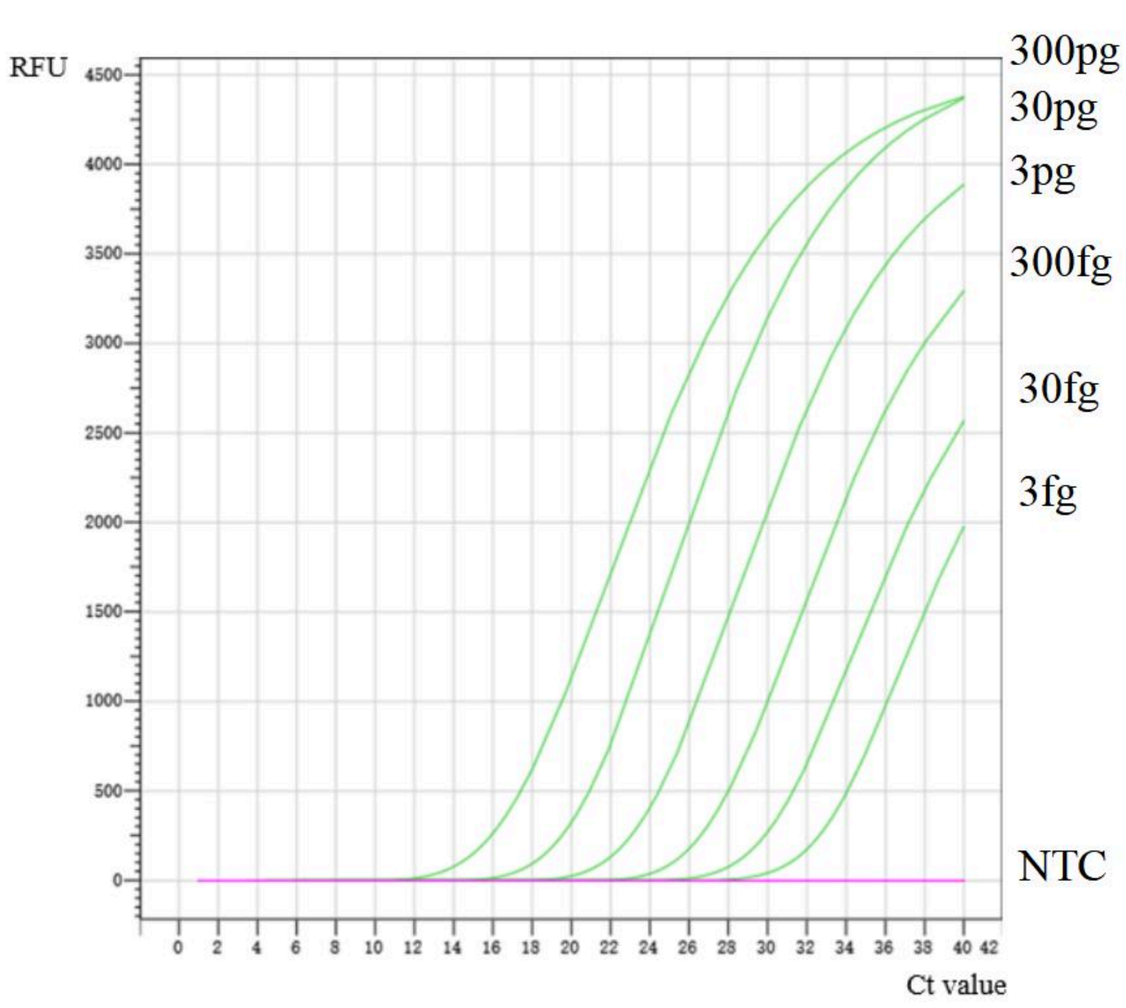
- NTC: CHO detector task = NTC
- NEC, ERC and samples wells: CHO detector task = Unknown

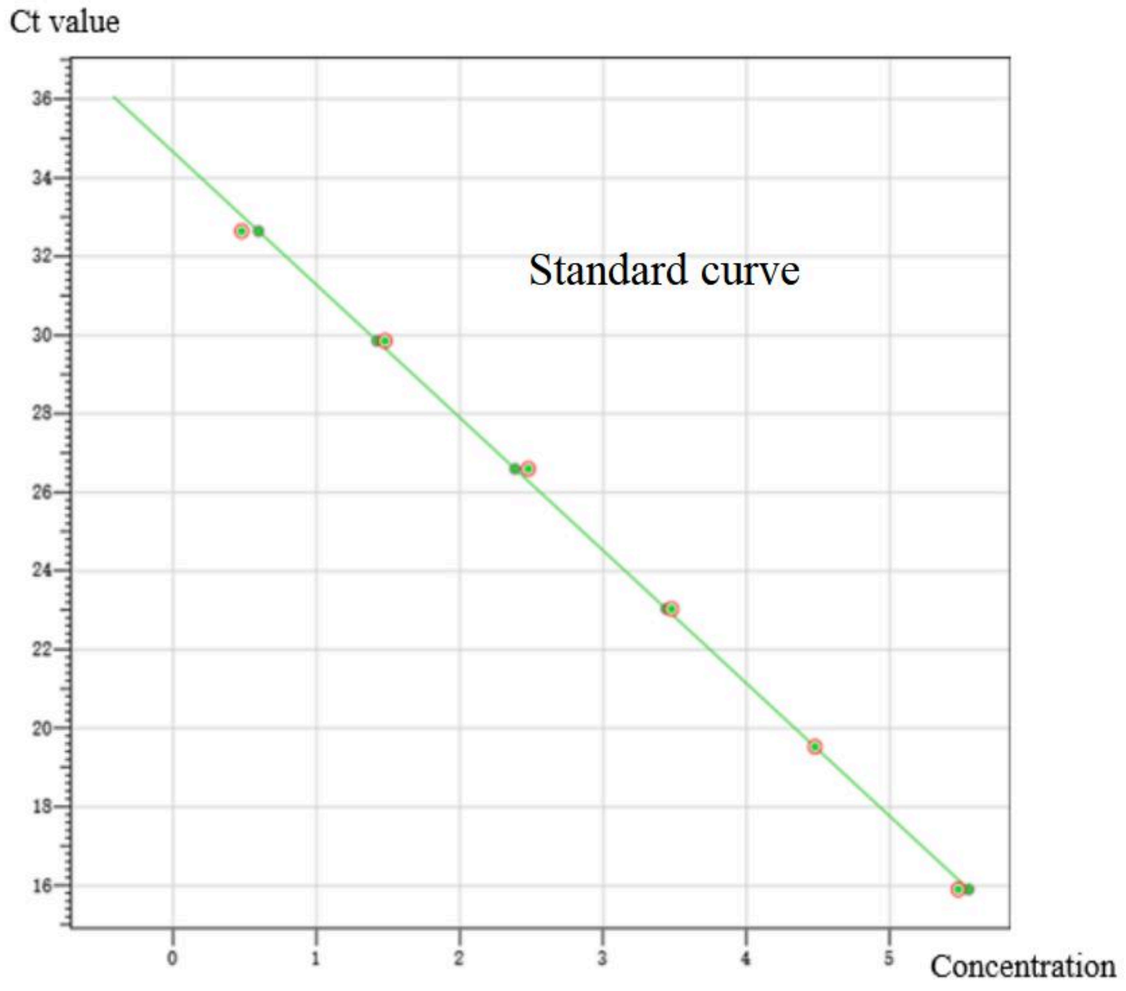
9.4. Select the Results tab, Standard Curve tab, then verify the Slope, Intercept, and R2 values.

9.5. Select the Report tab-Report, then review the mean quantity and standard deviation for each of the samples.

9.6. Select File-Export-Results. In the “Save as type” drop‑down list, select Results Export Files (*.csv), then click Save.

10. Typical curve





Project 1: FAM channel; Intercept: 34.65; Slope: -3.38;
Deviation: 0.010; Correlation coefficient: -0.999;
Efficiency: 97.73