resDetect™ CHO resDNA Quantitation Kit (qPCR)

Catalog Number：OPA-R004

Assay Tests: 100 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

IMPORTANT: Please carefully read this user guide before performing the experiment.

Product information

CHO resDNA Quantitation Kit is designed for quantitative detection of residual CHO DNA in biopharmaceuticals (Antibodies). Use the kit after extracting host cell DNA from test samples. For achieving the better DNA recovery, it is recommended to use the **resDetect™ resDNA Sample Preparation Kit (Magnetic Beads) (Cat. No. OPA-R005)** in combination.

Residual CHO DNA is quantified using a real-time polymerase chain reaction (PCR) assay. The PCR-based assay is sensitive and specific for DNA from CHO genome and not subject to detection of human or environmental DNA that might be introduced during sample handling. The kit was developed to meet the sensitivity requirements defined by WHO (10 ng CHO DNA per therapeutic dose).

To generate the standard curve used to quantitate the DNA in test samples, the CHO assays require six dilutions. Control DNA for standard curve generation is included in the kits. Linearity is demonstrated by analysis of standard DNA from CHO ranging from **3 fg/μL~300 pg/μL**. Limit of Detection is 1 fg/μL.

Contents and Storage

The kit contains sufficient reagents to run 100 PCR reactions each with a final reaction volume of 30 µL.

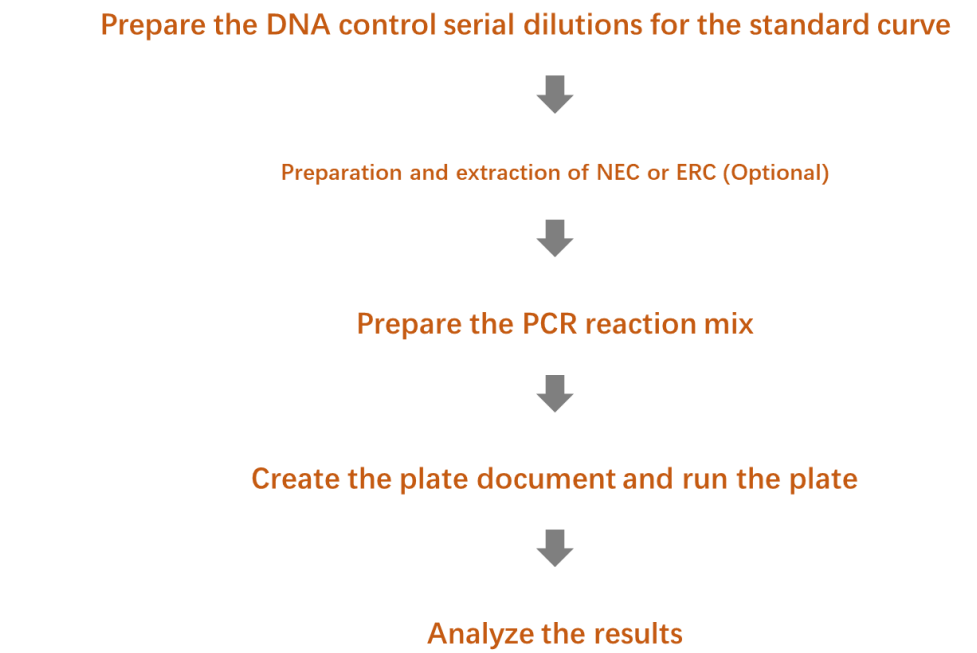
Contents	Colors	Amount	Storage
2×qPCR Master Mix		1.0mL×2	-30℃ to -15℃ **Note:** Primer&Probe Mix need protect from light.
CHO Primer&Probe Mix		700µL×1	
CHO DNA Control (3 ng/µL)		100µL×1	
Dilution Buffer		1.5mL×3	
DNase/RNase-Free Water		1.0mL×1	

The unopened kit is stable for 12 months from the date of manufacture if stored at -30°C to -15°C.

Required materials not supplied.

Instrument	Real-time PCR instrumentation
Consumables	96-Well Reaction Plate, Covers
	Nuclease-free, DNA-free aerosol-resistant pipet tips
	Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free) to prepare working solution, dilutions, and mixes

Workflow



NOTE: NEC=Negative Extraction Control； ERC=Extraction/Recovery Control

Prepare the DNA control serial dilutions for the standard curve.

Guidelines for standard dilutions

- Prepare the standard curve and the test samples in different areas of the lab.
- Use Low DNA-Binding microcentrifuge tubes and different sets of pipettors for test sample preparation and for standard curve preparation and aliquoting to avoid cross-contamination of test samples.
- Vortex each tube for **20-30 seconds** to ensure thorough mixing of the contents before proceeding with each dilution step.
- Briefly centrifuge to collect all the liquid at the bottom before making the next dilution.

Prepare the control serial dilutions

1. Label low DNA-binding microfuge tubes: **SD1, SD2, SD3, SD4, SD5, SD6**, **NTC**, where **SD** indicates serial dilutions and **NTC** indicates the no template control.
2. Add **30-40 µL** of DNase/RNase-Free Water to tube **NTC**. Put aside.
3. Add **90 µL** of Dilution Buffer to tubes **SD1, SD2, SD3, SD4, SD5, SD6**.
4. Remove the tube of CHO DNA control (3 ng/µL) from the freezer.
5. After the DNA thaws, vortex it gently for 20-30 seconds, then briefly centrifuge to collect the solution at the bottom.
6. Perform the serial dilutions:

   a. Add **10 µL** of the DNA control to the tube that is labeled **SD1**, then vortex thoroughly and briefly centrifuge.

   b. Transfer **10 µL** of the DNA from tube **SD1** to tube **SD2**, then vortex thoroughly and briefly centrifuge.

   c. Continue to transfer **10 µL** of DNA from the previous dilution tube to the next dilution tube until you add DNA to tube **SD6**. After each transfer, vortex thoroughly, then centrifuge briefly. Process of dilutions is shown in the following figure.
7. Store the **SD** tubes at 4°C for use within 24 hours.

Preparation and extraction of NEC and ERC (Optional)

Preparation and extraction of NEC

A Negative Extraction Control (NEC) omits any DNA template from a reaction. This control is used to monitor contamination during nucleic acid extraction. In cases where large numbers of DNA samples need extracted, it is recommended that negative extraction controls are included between the samples for testing.

1. Label low DNA-binding 1.5 mL microfuge tubes "NEC".
2. Add **100 µL** of 1X PBS (free of Mg^{2+} and Ca^{2+}) or 1×TE (pH7.0~pH8.0) to each tube.

When finished, extract DNA from the tubes according to the resDNA Sample Preparation Kit User Guide (Cat. No. OPA-R005), then quantify the extracted DNA in each tube using this Kit (Cat. No. OPA-R004) as described in the following section.

Preparation of Extraction/Recovery Control (ERC) (Optional)

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

1. For each sample, label low DNA binding 1.5 mL microfuge tubes "ERC".
2. Add the appropriate volume of test sample to each tube.
3. Add **20 µL** of DNA from tube **SD4 (300 fg/µL)** to each ERC tube, then vortex gently.

When finished, extract DNA from the tubes according to the resDNA Sample Preparation Kit User Guide (Cat. No. OPA-R005), then quantify the extracted DNA in each tube using this Kit (Cat. No OPA-R004) 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 ensure accurate results, the amount of CHO DNA control added to a test sample should be approximately two to three times 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.

Prepare the PCR reaction mix

Prepare serial dilutions of CHO DNA control from the same experiment to create a standard curve and to determine sample recovery rate.

1. Determine the number of controls and test samples whose DNA content you will quantify. Number of reaction wells is equal to three times the sum of **NTC, NEC, ERC, SD1-SD6, and test samples**.
2. Thaw reagents completely on ice, thoroughly mix reagent, and briefly centrifuge. Prepare a 2.0 mL tube for **Working Mix** (not add DNA template) using the reagents and volumes shown in the table below, thoroughly mix reagent, and briefly centrifuge. **IMPORTANT!** To compensate for pipetting losses, it is recommended that the **N** is equals to number of reaction wells plus 2 or 3.

Kit Reagents	Volume for 1 reaction (30-µL)	Volume for Working Mix
2×qPCR Master Mix	15 µL	15 µL×**N**
CHO Primer＆Probe Mix	5 µL	5 µL×**N**
DNA template	10µL	Add DNA template to each well separately, not as part of Working Mix
Total	30 µL	20 µL×**N**

3. Add **20 µL** Working mix to each well separately.
4. Add **10 µL** DNA template to the corresponding wells. **Final volume** of PCR reaction is **30 µL.** It is recommended that the above DNA samples (test samples, NTC, NEC, ERC, and SD) should be placed in different zones during the design and layout of the reaction wells to avoid cross contamination and inaccurate test results.

   NOTE: Set up a 96-well PCR plate using the example plate layout shown in the following page.
5. Seal the plate with an optical film, then quick-spin with a centrifuge rotor that is compatible with 96-well plates.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A									SD1	SD1	SD1	
B	S1	S1	S1		S1(ERC)	S1(ERC)	S1(ERC)		SD2	SD2	SD2	
C	S2	S2	S2		S2(ERC)	S2(ERC)	S2(ERC)		SD3	SD3	SD3	
D	S3	S3	S3		S3(ERC)	S3(ERC)	S3(ERC)		SD4	SD4	SD4	
E									SD5	SD5	SD5	
F									SD6	SD6	SD6	
G	NEC	NEC	NEC									
H									NTC	NTC	NTC	

S=Sample； NTC=No Template Control； NEC=Negative Extraction Control；

ERC= Extraction/Recovery Control

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

Create the plate document and run the plate

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

1. Create a new experiment, enter the experiment name in the Plate name field.
2. Select the **Quantitation Standard Curve** mode, **TaqMan** reagents, and **Standard** mode.
3. In the Plate Setup, enter the Target Name. Select **FAM** in the Reporter Dye drop-down list. Select **(None)** in the Quencher Dye drop-down list. Select **ROX** in the Passive Reference Dye drop-down list.
4. Set up the standard curve as shown in the Plate Layout. Assign the tasks and the enter the appropriate Quantity for each set of triplicates. (SD1-SD6, 300000, 30000, 3000, 300, 30, 3 fg/μL)
5. Set up the test samples and controls as shown in the Plate Layout.
6. Set up the qPCR reaction program according to following Table.
7. Select the reaction volume to 30 μL, click "Start Run" in the Run interface to start the qPCR run, and analyze the results after completion.

Step	Temperature	Cycles	Time	Signal Collection
UDG	37℃	1×	2 mins	No
Initial Denaturation	95℃	1×	10 mins	No
Denaturation	95℃	40×	15s	No
Extension	60℃		40s	**Yes**

Analyze the results

After the qPCR run is finished, use the general procedure to analyze the results. The threshold value for ABI7500 is set to 0.2, and analysis is performed using the Auto Baseline setting. For other instruments, the setting of parameters should be adjusted according to the specific instrument user guide and software version. For more information, refer the Getting Started Guide that is supplied with the specific analysis software. The acceptance criteria of results are shown in the following list:

1. The Standard curve: $R^2 \geqslant 0.98$, Eff%=90-110%.
2. The detection result of NTC should be undetermined or Ct value > 35.
3. The Ct value of NEC should be greater than the Ct value of the SD6.
4. Ct values should be consistent with replicates, the differences between the Ct values of replicates is less than 0.5.
5. The spike recoveries of test samples and controls should be between 50%-150%.

Note: Calculate the concentration of the test sample (pg/μL or fg/μL) based on the standard curve, the Ct value of test sample is only valid for concentration calculation within the assay range of standard curve. When Ct values is outside the range of standard curve, do not use the data to calculate the concentration of test sample.

Typical data

- Highly sensitive quantitation using the CHO resDNA Quantitation Kit (qPCR).

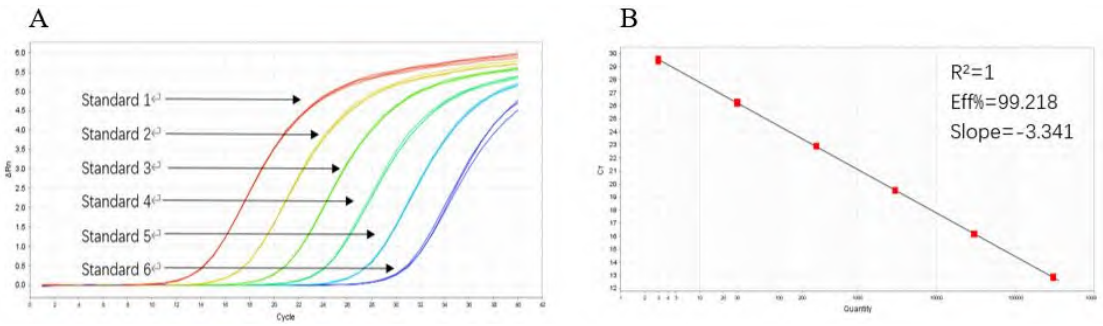


Figure 1. High sensitivity and broad dynamic range using the CHO resDNA Quantitation Kit. (A) Typical analysis results obtained with Standard 1 (300 pg/μL) to 6 (3 fg/μL). (B) The standard curve of the 10-fold dilution series. PCR efficiency should be 90-110%.

- High specificity: No cross-reactivity with unrelated DNA

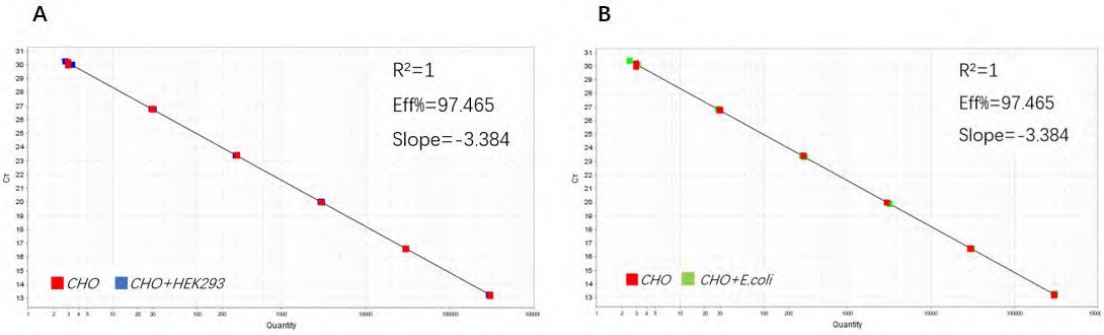


Figure 2. Assay specificity. Standard curves generated using 10-fold serial dilution of CHO Control DNA (included in the kit). A, unrelated DNA is HEK293 DNA; B, unrelated DNA is *E. coli* DNA.

- High consistency: Consistent performance across the expected range of DNA fragment sizes

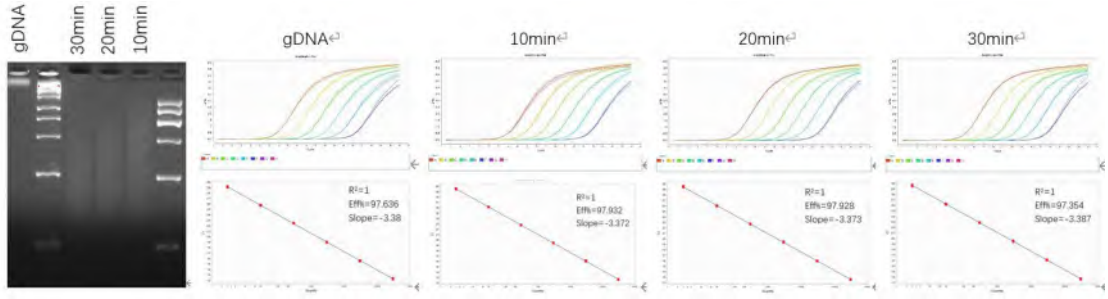


Figure 3. Consistent quantitation across a broad range of fragment sizes. Standard curves were generated using a 10-fold serial dilution of gDNA and fragmented DNA. Fragmented DNA was generated by sonicating total CHO genomic DNA (10min, 20min, 30min). Fragmentation of the DNA was confirmed by agarose gel analysis.

- Precision

To assess the repeatability of the kit, two different levels samples (3 pg/μL, 30 fg/μL) were tested 10 times. Acceptable criteria：CV value should be under 15%.

Table 1 Repeatability of CHO residual DNA quantitative assay

CHO DNA	Mean (fg/μL)	n	CV%
3 pg/μL	2813.959	10	4.11%
30 fg/μL	26.147	10	2.79%

To assess the intermediate precision of the kit, three samples of known concentration (3 pg/μL, 30 fg/μL, 3 fg/μL) were tested 9 times in separate assays.

Table 2 Intermediate precision of CHO residual DNA quantitative assay

Tester	Sample 1 (3000fg/μL)	Sample 2 (30fg/μL)	Sample 3 (3fg/μL)
Tester 1	3037.46	30.80	2.97
	3132.02	32.05	3.25
	3218.50	31.85	2.81
Tester 2	2975.61	28.55	2.87
	3022.23	29.62	3.14
	3101.48	29.15	2.78
Tester 3	2986.88	29.38	2.73
	3086.4	30.9	2.97
	3007.19	29	2.95
Mean (fg/μL)	3063.09	30.14	2.94
CV%	2.58%	4.27%	5.76%

- Stability

  After six cycles of freeze-thaw process, the performance of CHO resDNA Quantitation Kit is not observed significant changes.

Table 3 Results of Freeze-thaw testing

Cycles	0	3	5	8
Eff%	98.756	97.449	98.87	100.219
R^2	1	1	1	1
Slope	-3.352	-3.385	-3.349	-3.317

After long-term 2-8 °C stability testing, no significant changes are observed.

Table 4 Results of Long-term 2-8 °C stability testing

Days	0	3	7	10
Eff%	98.756	99.772	96.533	96.85
R^2	1	1	1	1
Slope	-3.352	-3.327	-3.408	-3.4