ResiDNA Precise Quantitative CHO DNA Detection Kit

RD102

Vazyme

Instruction for Use

Version 23.1

Vazyme

Contents

01/Product Description

ResiDNA Precise Quantitative CHO DNA Detection Kit is a probe-based qPCR kit that quantitatively detects residual genomic DNA from CHO host cells. This kit is applicable for the detection of residual CHO DNA in the intermediate, semi-finished and finished products of various biological products and pharmaceuticals. It contains CHO DNA quantitative reference material (CHO DNA Template, 30 ng/μl), which has the characteristics of strong specificity, high sensitivity (detection of fg/μl level), rapid detection, good stability and convenient operation. This kit can be used with ResiDNA Hunter Residual DNA Sample Preparation Kit (Vazyme #RD101) for accurate detection of residual DNA in CHO host cells.

02/Components

	Components	RD102-01 (100 rxns)
■	2 × CHO qPCR Mix[a]	2 × 750 μl
	10 × CHO Primer & Probe Mix	300 μl
■	CHO DNA Template (30 ng/μl)	40 μl
□	DNA Dilution Buffer	7 × 1 ml
■	Negative Control	1 ml

a. It contains dNTP Mix, Mg^{2+}, hot-start Taq DNA polymerase, universal reference dye, etc. This component needs to be protected from light after unpacking.

03/Storage

Store at -30 ~ -15℃ and transport ≤0℃.

04/Application

It is applicable for the detection of residual CHO DNA in biological products expressed from CHO cells.

05/Applicable Instruments

Including but not limited to:

ABI QuantStudio 3, ABI QuantStudio 5, ABI 7500, ABI StepOnePlus, Bio-Rad CFX 96, Roche LightCycler 96, Bioer Linegene 9600.

06/Notes

For research use only. Not for use in diagnostic procedures.

07/Experiment Process

1. Dilution and standard curve preparation of CHO DNA Template (30 ng/μl)

   Use the DNA Dilution Buffer provided in the kit to carry out serial dilutions of CHO DNA Template, the concentrations are 300 pg/μl, 30 pg/μl, 3 pg/μl, 300 fg/μl, 30 fg/μl and 3 fg/μl. The detailed steps are as follows:

   a. Thaw CHO DNA Template (30 ng/μl) and DNA Dilution Buffer on ice, and then mix gently. Centrifuge for 5 sec.

   b. Dilute CHO DNA Template (30 ng/μl) in 1.5 ml Nuclease-free centrifuge tubes according to Table 1 and mark them as SD 1, SD 2, SD 3, SD 4, SD 5, SD 6.

   c. Mix CHO DNA Template (30 ng/μl) and DNA Dilution Buffer thoroughly. Vortex for 30 sec, and then centrifuge for 10 sec.

Table 1. Serial Dilutions of CHO DNA Template

Tube	Dilution	Final concentration
SD 1	10 μl CHO DNA Template + 990 μl DNA Dilution Buffer	300 pg/μl
SD 2	50 μl SD 1 + 450 μl DNA Dilution Buffer	30 pg/μl
SD 3	50 μl SD 2 + 450 μl DNA Dilution Buffer	3 pg/μl
SD 4	50 μl SD 3 + 450 μl DNA Dilution Buffer	300 fg/μl
SD 5	50 μl SD 4 + 450 μl DNA Dilution Buffer	30 fg/μl
SD 6	50 μl SD 5 + 450 μl DNA Dilution Buffer	3 fg/μl

▲ The linear range of the standard curve can be adjusted according to the needs, and the recommended range is 3 fg/μl - 300 pg/μl.

▲ Diluted SD 1 can be stored at -20℃ for 6 months, and SD 2 - SD 6 can be stored at -20℃ for 1 week;

▲ Pay attention to the accuracy of volume during serial dilution. Vortex for 30 sec to ensure that the template is mixed thoroughly and exhibits a good linear relationship.

2. Preparation of Extraction Recovery Control (ERC)

   Set the CHO DNA loading concentration in the ERC as needed. Take the preparation of ERC with 3 pg CHO DNA as an example:

   a. Add 100 μl of the sample to be tested into a 1.5 ml Nuclease-free centrifuge tube. Add 10 μl SD 4, mix thoroughly, and mark it as ERC.

   b. Perform sample pretreatment on ERC and the same batch of samples to be tested to prepare ERC purified solution.

3. Preparation of Negative Extraction Control (NC)

   a. Add 100 μl of sample dilution buffer into a 1.5 ml Nuclease-free centrifuge tube and mark it as NC.

   b. Perform sample pretreatment on NC and the same batch of samples to be tested to prepare NC purified solution.

4. Prepare the following mixture in a qPCR tube:

Components	Volume	
2 × CHO qPCR Mix	15 μl	■
10 × CHO Primer & Probe Mix	3 μl	
Negative Control	2 μl	■
DNA Template[a]	10 μl	
Total	30 μl	

a. DNA Template includes standard curve (SD 1 - SD 6), NTC (Negative Control), NC (Sample Dilution Buffer), ERC purified solution and samples to be tested;

▲ Vortex the mixture to mix thoroughly, remove air bubbles, then centrifuge it briefly to the bottom of the tube.

5. Reaction Program

Stage 1	Initial Denaturation	Rep: 1	95℃	10 min
Stage 2	Cycling Reaction	Reps: 40	95℃	15 sec
			60℃	1 min*

*Acquisition of FAM channel signal.

6. Data calculation and analysis (Using ABI QuantStudio 3)

a. Use the default baseline and threshold. Export the data and calculate average C_T values. The following table is obtained:

Table 2. The average C_T values of SD 1 - SD 6

Gradient	C (pg/μl)	Log C	Avg. C_T	$\triangle C_T$
SD 1	300	2.47712125	15.267	—
SD 2	30	1.47712125	18.634	3.37
SD 3	3	0.47712125	21.973	3.34
SD 4	0.3	-0.52287875	25.307	3.33
SD 5	0.03	-1.52287875	28.754	3.45
SD 6	0.003	-2.52287875	32.089	3.34

▲ $\triangle C_T$ should be between 3.1 - 3.6

b. Draw a standard curve using Log C and the average C_T value:

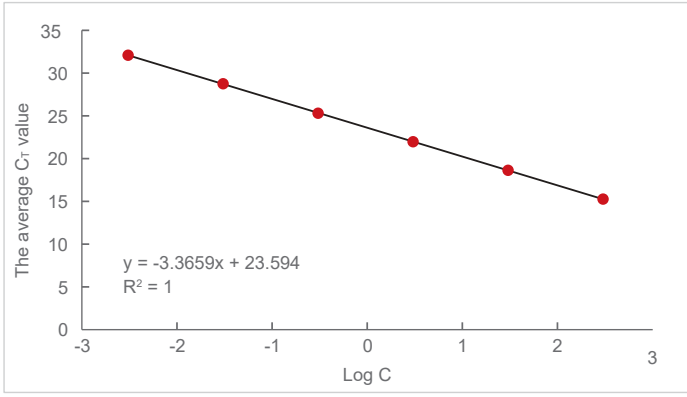

Fig 1. Standard curve

c. Concentration of the sample to be tested:

Substitute the average C_T value of the sample to be tested into the standard curve to obtain Log C (to be tested), then the concentration of the sample to be tested can be calculated.

08/FAQ & Troubleshooting

◇ Amplification efficiency deviates from the range of 90% - 110%

① It indicates that the reaction system may be contaminated, if C_T(NTC) - C_T(SD 6) <3 or C_T(SD 6) - C_T(SD 5) <3.1 and the calculated amplification efficiency exceeds 100%. It is recommended to confirm effective C_T values according to C_T(NTC), discard contaminated C_T values, and then draw a standard curve.

② The incorrect setting of baseline baseline will increase the C_T(SD 1) value, thereby affecting the calculation of amplification efficiency. Manually adjust the baseline to 1 - 3 cycles.

③ Inaccurate pipetting volume.

◇ R^2<0.99

① Inaccurate pipetting volume.

② All reagents should be fully thawed and mixed thoroughly before use.

◇ Uneven distribution of standard amplification curve

① C_T(SD 2) - C_T(SD 1) <3.1 indicates that the baseline is not set correctly. Manually adjust the baseline to 1 - 3 cycles.

② $\triangle C_T$ >3.6 between SD 1 and SD 6 indicates low amplification efficiency. Make sure that all reagents are fully thawed and mixed thoroughly before use.

③ Confirm that all component concentrations and reaction program are correct.

◇ Abnormal shape of amplification plot

① Rough amplification plot: The signal is too weak and generated after system correction. Increase template concentration and retry.

② Broken or downward amplification plot: The template concentration is too high and the baseline endpoint is greater than C_T value. Reduce the baseline endpoint (C_T value - 4) and repeat data analysis.

③ Amplification plot goes downward suddenly: There are bubbles remaining in the reaction tube. Pay attention to centrifugation when processing samples and carefully check the reaction tube for any remaining bubbles before performing reaction.

◇ Amplification observed in negative control

① Reaction system contamination: Replace with new mix, ddH_2O and primers to repeat the experiment. The reaction system should be prepared in clean bench to reduce aerosol contamination.

◇ Standard curve linearity is poor for absolute quantification

① CHO DNA Template degradation: Prepare the CHO DNA Template again and repeat the test.

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