CHO Cellular Residual DNA Detection Kit

For Research Use Only.

■ Introduction

CHO Host Cell Residual DNA Detection Kit, which is produced by Genebio, can be used as quantitative determination of CHO cell residual DNA in biological products (e.g. recombinant protein, antibodies, vaccines, etc) by quantitative Polymerase Chain Reaction with TaqMan fluorescent probes. The kit has the characteristics of high speed and high specificity.

The detection method can be traced back to China Pharmacopoeia method.

■ Kit components

Table 1. Kit components of CHO Cell Residual DNA Detection Kit

Name	Specification	Tube Number	Storage Condition
CHO DNA Positive Control	30ng/μl, 50μl	1	-25℃~-15℃
TaqMan qPCR Master Mix	1ml	2	-25℃~-15℃, away from light
DNA Dilution Buffer	1.5ml	3	-25℃~-15℃

Specification: 100 reactions.

Period of validity: 24 months under specified storage conditions

Transportation and storage: Store at -20℃ for long term storage and use dry ice to keep it cold during transportation.

Operating steps:

■ Preparation of CHO DNA Positive Control standard curve samples

The concentration of CHO DNA Positive Control is marked on the product label. Check the actual concentration before dilution. Dilute CHO DNA Positive Control with DNA dilution buffer in kit by gradient dilution. The diluted concentration is as follows: 3000pg/μl, 300pg/μl, 30pg/μl, 3pg/μl,0.3pg/μl, 0.03pg/μl, 0.003pg/μl.

The specific operating steps are as follows:

1. *Take out and thaw CHO DNA Positive Control and DNA Dilution Buffer from -20℃ freezer. Vortex and mix slightly after completely melted then centrifuge rapidly for 2-5 seconds.*
2. *Take out 7 low retention centrifuge tubes and mark them as ST0, ST1, ST2, ST3, ST4, ST5 and ST6. Prepare CHO DNA standard samples according to the following table. Mix with vortex mixer then centrifuge rapidly for 2-5 seconds before starting the next dilution step. Store the diluted standard samples at 2~8℃. Freshly prepared samples are more recommended.*

Table 2. Dilution of CHO DNA Positive Control

Dilution Tube	Dilution Steps	Concentration (pg/μl)
ST0	10μl DNA Positive Control + 90μl DNA Dilution Buffer	3000
ST1	10μl ST0 + 90μl DNA Dilution Buffer	300
ST2	10μl ST1 + 90μl DNA Dilution Buffer	30
ST3	10μl ST2 + 90μl DNA Dilution Buffer	3
ST4	10μl ST3 + 90μl DNA Dilution Buffer	0.3
ST5	10μl ST4 + 90μl DNA Dilution Buffer	0.03
ST6	10μl ST5 + 90μl DNA Dilution Buffer	0.003

The melted but unused DNA Dilution Buffer can be temporarily stored at 2-8℃.

■ Preparation of Extraction/Recovery Control (ERC)

Spike CHO DNA positive control in ERC as required. (It is suggested that the spike-in amount should be 2 to 30 times of the historical sample data). For example, the specific operating steps of 30pg DNA spiked in ERC sample are as follows:

1. *Add 100μl of testing sample into 1.5mL low retention centrifuge tube.*
2. *Add 10μl of ST3, vortex and mix well then mark the centrifuge tube as ERC.*
3. *ERC should be extracted synchronously with the same batch of testing samples, then ERC eluent can be harvested.*

■ Preparation of Negative Control Sample(NCS)

The specific operating steps of NCS sample are as follows:

1、Add 100μl of sample matrix (or DNA Dilution Buffer) into 1.5mL low retention centrifuge tube and mark the tube as NCS.

2、NCS should be extracted synchronously with the same batch of testing samples then NCS eluent can be harvested.

■ qPCR reaction systems

1. Calculate the required number of qPCR wells according to the following formula:

Number of qPCR wells=(6 standard curve samples +1 BLK + 1 NCS + SAM + ERC)×3.

Table 3. presents the composition of each reaction.

Table 3. Sample loading example for each reaction

Standard Curve	20μl TaqMan qPCR Master Mix + 10μl ST1/ST2/ST3/ST4/ST5/ST6
BLK	20μl TaqMan qPCR Master Mix + 10μl DNA dilution buffer
NCS	20μl TaqMan qPCR Master Mix + 10μl NCS eluent
SAM	20μl TaqMan qPCR Master Mix + 10μl testing sample eluent
ERC	20μl TaqMan qPCR Master Mix + 10μl ERC eluent

■ Sample loading of qPCR reaction

1. *Take out 96-well PCR plate and add 20μl TaqMan qPCR Master Mix into each well.*
2. *According to Table 4, add BLK, NCS, SAM, ERC, and then add 10μl of ST1, ST2, ST3, ST4, ST5 and ST6 DNA standard solution in order. Triplicated wells were made for each sample.*
3. *Seal the plate with adhesive membrane, mix well and centrifuge for qPCR reaction.*

Table 4. Sample loading example for 96-well plate

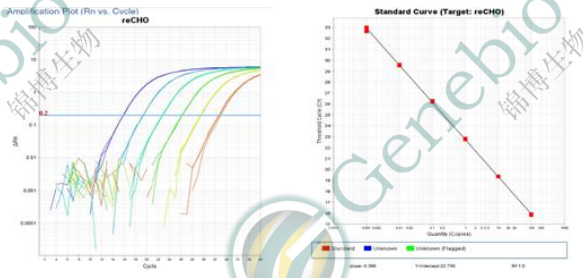
	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	BLK	BLK							ST6	ST6	ST6
B	NCS	NCS	NCS							ST5	ST5	ST5
C	SAM1	SAM1	SAM1							ST4	ST4	ST4
D	SAM2	SAM2	SAM2							ST3	ST3	ST3
E	SAM3	SAM3	SAM3							ST2	ST2	ST2
F	ERC1	ERC1	ERC1							ST1	ST1	ST1
G	ERC2	ERC2	ERC2									
H	ERC3	ERC3	ERC3									

■ qPCR instrument running program settings

Take Applied Biosystems® 7500 Fast qPCR instrument as an example.

1. *Create a blank new program and select the absolute quantitative detection template.*
2. *Create a new detection probe, name it as "CHO-DNA", select the reporter fluorophore as FAM, the quencher fluorophore as TAMRA, and the detection reference fluorescence as ROX.*
3. *Set up a two-step reaction program.*
4. *Pre-denaturation at 95℃ for 10 min, 95℃ for 15s, 60℃ for 1 min with 40 cycles. The reaction volume is 30μl.*

■ Standard curve PCR results:



■ Results

1. Calculation of residual DNA amount in testing sample as follows:

$$\text{Exogenous DNA residues(pg/mg)} = \frac{\text{Dilution factor} \times \text{Average value of testing sample (pg/μl)} \times \text{Elution volume(μl)}}{\text{Protein concentration of testing sample (mg/ml)} \times \text{extraction volume of testing sample(ml)}}$$

Calculation of variation coefficient of triplicated wells:

$$\text{Coefficient of Variation(CV\%)} = \frac{\text{Standard deviation of DNA content in duplicated wells}}{\text{DNA average value in duplicated wells}} \times 100\%$$

For example, if the result of the DNAsample is close to the lower limit of detection (ST6) (Ct value ± 2 cycles) or the sample Ct value is higher than ST6 Ct value, the coefficient of variation is not calculated.

2. Calculation of ERC recovery rate

DNA concentration of ERC can be determined according to PCR standard curve equation and Ct value of ERC. The recovery rate can be calculated as follows:.

$$\text{ERC recovery rate\%} = \frac{(\text{Concentration of ERC (pg/μl)} - \text{Concentration of testing sample(pg/μl))} \times \text{Elution volume(μl)}}{\text{Spiked DNA Amount(pg)}} \times 100\%$$

Note:

- When using chemicals, must wear appropriate lab coats, disposable gloves and goggles.
- If reagent can not be used up at one time, please store it in the refrigerator at -20℃
- If reagent is accidentally spilled into your eyes, mouth and nose, please rinse with plenty of water immediately.
- Please stop using the kit if tubes are damaged or labels are unclear.

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