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# CHO Host Cell Residual DNA Detection Kit (qPCR fluorescence probe method)

**Product Number: RDK104**

## Shipping and Storage

Under specified storage conditions for 2 years, please refer to the reagent kit label for details.

## Components

| Component | 50T | 100T | Store |
|---|---|---|---|
| CHO qPCR Mix | 700μL | 2×700μL | -20℃ |
| CHO Primer&Probe Mix | 110μL | 220μL | -20℃, light-proof |
| CHO DNA quantification reference substance (30ng/μL) | 25μL | 50μL | -20℃ |
| DNA diluent | 2×1.2mL | 4×1.2mL | -20℃ |

Note: This kit does not contain ROX reference dye. If your instrument requires the addition of ROX reference dye, it needs to be purchased separately.

## Description

The CHO Host Cell Residual DNA Detection Kit is a specialized reagent kit designed for the quantitative analysis of CHO host cell residual DNA content in various biological products, including intermediates, semi-finished products, and finished goods. This kit employs the probe-based fluorescence quantitative PCR principle to accurately measure the residual DNA content in samples. It features high specificity, rapid detection, stable performance, and a minimum detection limit capable of reaching the fg level.

The CHO DNA quantitative reference material included in this kit is traceable to national standard reference materials, enabling accurate quantification of CHO residual DNA in samples.

## Applicable instruments

1. Thermo Scientific: ABI 7500, QuantStudio ™ 5, ABI StepOnePlus
2. Bio-Rad: CFX96
3. Shanghai Hongshi: SLAN-96S.

## Required equipment

1. Instruments: pipette, vortex mixer, instant centrifuge, qPCR instrument.
2. Materials: EP tube, PCR tube, eight tube, pipette tip, etc.

## Protocol

**1. Dilution of CHO DNA Quantitative Reference and Preparation of Standard Curve.**

The concentration of CHO DNA quantification reference substance is labeled on the tube wall label. Please confirm before diluting.

Using CHO DNA quantification reference substance at an initial concentration of 30ng/μL, the reference substance was diluted in a gradient using DNA diluent, with dilution concentrations of 300ng/μL in sequence pg/μL, 30pg/μL, 3pg/μL, 300fg/μL, 30fg/μL, 3fg/μL. The specific operation is as follows:

1.1. Add 250μL anhydrous ethanol, mix well without centrifugation.

1.2. Melt the DNA quantification reference substance and DNA dilution solution in the reagent kit at low temperature or room temperature. After complete melting, gently shake and mix, and centrifuge instantly for 3-5 seconds.

1.3. Take 7 clean 1.5mL centrifuge tubes and label them as 3ng/μL, ST ①, ST ②, ST ③, ST ④, ST ⑤, ST ⑥.

1.4. Add 90μL of DNA dilution solution and 10μL of DNA quantification reference substance (30 ng/μ L) into a centrifuge tube labeled as 3ng/μL, dilute to 3ng/μL, shake and mix instantly, then centrifuge rapidly for 3-5 seconds, repeat 2-3 times to ensure thorough mixing of the quantification reference substance and DNA dilution solution. This concentration can be stored at temperatures below -20℃ for a short period of time (not exceeding 1 month), and repeated freezing and thawing should be avoided during use.

1.5. Add 90μL of DNA diluent to tubes ST ①, ST ②, ST ③, ST ④, ST ⑤, and ST ⑥ respectively, and then perform gradient dilution. The dilution method is as follows:

| Dilution tube | Dilution ratio | Final concentration |
|---|---|---|
| ST① | 10μL 3ng/μL+90μL DNA diluent | 300pg/μL |
| ST② | 10μL ST ①+90μL DNA diluent | 30pg/μL |
| ST③ | 10μL ST ②+90μL DNA diluent | 3pg/μL |
| ST④ | 10μL ST ③+90μL DNA DNA diluent | 300fg/μL |
| ST⑤ | 10μL ST ④+90μL DNA diluent | 30fg/μL |
| ST⑥ | 10μL ST ⑤+90μL DNA diluent | 3fg/μL |

Note: Melted and unused DNA diluent can be stored at 2-8℃. If not used for a long time, please place it at -20℃.

When using for the first time, the DNA quantification reference sample should be packaged and stored to reduce the number of repeated freeze-thaw cycles and avoid contamination.

The concentration point of the standard curve can be selected based on the actual verification results, and if necessary, the linear range can be appropriately expanded or reduced.

Each gradient dilution requires slight oscillation and instantaneous centrifugation before suction to ensure complete mixing of the template.

Use a nozzle with a filter element and replace it every time. Pay attention to standardized operation to avoid direct cross contamination between pipes.

**2. Preparation of different control samples.**

2.1. Preparation of Extraction Recovery Control (ERC) for sample spiked recovery quality control.

Set the concentration of CHO DNA standard in ERC as needed (taking the preparation of CHO DNA samples with 3pg/μL as an example). The specific operation is as follows:

2.1.1. Take 50μL of the test sample (TS) and add it to a 1.5mL clean centrifuge tube. Then add 50μL of ST ③, mix well, and mark it as standard recovery ERC;

2.1.2. Prepare a purified solution of spiked recovered ERC by performing sample pretreatment together with the same batch of test samples.

2.2. Preparation of Standards Recovery Control (SRC) for standard product recovery (optional)

Set the concentration of CHO DNA standard in SRC as needed (taking the preparation of CHO DNA sample with 3 pg/μ L as an example), and the specific operation is as follows:

2.2.1. Take 100μL of ST ③ and add it to a 1.5mL clean centrifuge tube, marking it as the standard recovery SRC;

2.2.2. Sample pretreatment is carried out together with the standard recovery SRC and the same batch of test samples to prepare a purified solution of standard recovery SRC.

2.3. Preparation of negative quality control NCS

According to the experimental setting of negative quality control, the specific operation is as follows:

2.3.1. Take 100μL of sample recovery solution (or DNA diluent) and add it to a 1.5mL clean centrifuge tube, labeled as negative quality control NCS;

2.3.2. The negative quality control NCS is subjected to sample pretreatment together with the same batch of test samples to prepare a purified negative quality control NCS solution.

2.4. Preparation of template free control NTC

According to the experimental setup, template free control NTC does not require sample pretreatment and can be prepared during the preparation and sample addition stages of qPCR reaction solution.

**3. Preparation and Sampling of qPCR Reaction Solution.**

3.1. Calculate the required number of reaction wells based on the standard curve to be tested and the number of samples to be tested. Typically, each sample requires 3 replicate wells.

Number of reaction wells=(6 concentration gradient standard curves+1 negative control NCS+1 template free control NTC+n sample spiked recovery control ERC+n test samples+standard recovery control SRC (optional)) × 3

It is usually recommended to perform ERC on each test sample. If several test samples are the same product, only one can be performed.

3.2. According to the number of reaction wells, mix the components except for the template into a Mix reaction solution. Place each reagent on ice or melt at low temperature, gently shake and mix well, and add the sample.

Mix reaction solution=(number of reaction wells+2) × 13μL CHO qPCR Mix+(number of reaction wells+2) × 2μL CHO Primer&Probe Mix (containing a loss amount of 2 wells, which can be appropriately increased if there are many test samples)

| Component | Volume |
|---|---|
| CHO qPCR Mix | 13μL |
| CHO Primer&Probe Mix | 2μL |
| DNA template | 10μL |
| Total | 50μL |

3.3. After thoroughly mixing the prepared Mix reaction solution, divide it and add the DNA template. After the sample is added, seal the tube, centrifuge at low speed, mix well, and then centrifuge at low speed to completely mix the reaction solution. If there are bubbles, they should be completely removed.

The following table shows an example of the arrangement of 96 hole plates.

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

Note: This example shows the detection of CHO DNA residue in 5 samples using this kit.

Including: CHO DNA standard curves with 6 concentration gradients (ST ① - ST ⑥), 1 template free control NTC, 1 negative quality control NCS, 5 sample spiked recovery quality controls ERC, 5 test samples S, and 1 standard recovery quality control SRC. At least 3 replicate wells should be made for each sample.

**4. Amplification program parameter settings**

Set the amplification program on the fluorescence quantitative PCR instrument: set the reaction volume to 25 μ L and FAM channel.

| Steps | Temperature | Time | Cycle |
|---|---|---|---|
| Pre-denaturation | 95℃ | 5min | 1 |
| Denaturation | 95℃ | 20sec | 40 |
| Annealing/extension (collecting fluorescence) | 60℃ | 30sec | 40 |

**Taking ABI 7500 v2.3 as an example**

4.1. Create a blank new program and select the absolute quantitative detection template. Select 7500 (96 Wells), Quantity Standard Curve, TaqMan Reagents on the Experiment Properties interface

4.2. Set up detection probes and samples on the Plate Setup interface. Create one detection probe (Define Targets) in the "Define Targets and Samples" panel, named "CHO DNA", select the reporting fluorescent group as "FAM", and the quenching group as "none"; Create Define Samples, ST ① - ST ⑥ NTC、NCS、ERC、S、SRC。

4.3. In the "Assign Targets and Samples" panel, set the "Task" column of the standard curve to "Standard" (), and assign values of "300000", "30000", "3000", "300", "30", and "3" in the "Quantity" column (i.e. the reference concentration added to each well, in fg/μ L). Select "ST ①", "ST ②", "ST ③", "ST ④", "ST ⑤", and "ST ⑥" in the corresponding sample column; Set the "Task" column of the NTC hole without template comparison to "Negative Control()", and select "NTC" in the corresponding sample column; Set the "Task" column for negative quality control NCS well, sample spiked recovery quality control ERC well, test sample well, and standard recovery quality control SRC well (optional) to "Unknown" (), and select "NCS", "ERC", "S", and "SRC" for the corresponding sample column.

4.4. In the "Run Method" panel, set the reaction program according to the amplification program parameters, with a reaction volume of 25μL.

4.5. Click on "START" in the upper right corner to start the program.

**5. Result Analysis**

5.1. Taking ABI 7500 v2.3 as an example

5.2. Enter the Analysis section

In the Amplification Plot interface, the system will automatically provide the Threshold, which can be manually adjusted to the appropriate position as needed. After completion, click "Analyze". You can check whether the amplification curve shape is normal in the "Multiple Plots View". If one of the three replicates has a significant difference, it can be discarded.

5.3. In the Standard Curve interface, read the R2, amplification efficiency (Eff%), slope, intercept (Y-Inter), etc. of the standard curve.

5.4. Normal standard score: R2>0.99; The amplification efficiency is between 90% and 110%.

5.5. In the "View Well Table" panel on the right, the "Quantity" column can read the detection values of different samples, in units of fg/μL. Subsequently, the units can be converted to the required units in the detection report.

5.6. The test results of NTC without template control should be Undetermined or Ct value ≥ 35.00, or specific standards should be set based on the laboratory's own validation results.

5.7. The Ct value of negative quality control NCS should be greater than the mean Ct value of the lowest concentration of the standard curve.

5.8. If the recovery rate of ERC is outside 50% -150%, while the recovery rate of SRC is within 50% -150%, there may be interference in the sample that affects the recovery rate of ERC.

## Note

1. Please read this manual carefully before testing and follow the instructions for standardized operation.
2. Each component is melted on ice or at low temperatures to avoid excessive temperature. Before use, please shake and mix well and centrifuge instantly to the bottom of the tube.
3. The sample addition and liquid preparation process should be carried out in separate areas of the ultra clean bench to avoid prolonged time. If the time is too long, please try to perform it on ice.
4. After using the reagent, place it under the recommended storage conditions as soon as possible to avoid prolonged storage at room temperature or high temperature.

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5. The consumables used for EP tubes, PCR tubes, and gun heads must be sterile and free of nucleases.