Cat.: BP-QN06-100

CHO residual DNA detection kit (qPCR) V01

Package Specification

100 Reactions / Kit

Intended Use

This kit is used for the quantitative detection of residual CHO host cell DNA in the intermediates, semi-finished products and finished products of various biological products and pharmaceuticals.

Product Introduction

This kit adopts the PCR-fluorescent probe method, and designs primers and probes for the conserved sequences of CHO cells to realize quantitative detection of CHO residual DNA in samples. The kit contains an exogenous internal standard, which can participate in extraction and amplification together with the sample to monitor whether the extraction and amplification are abnormal and prevent the occurrence of false negatives. The kit is equipped with UDG enzyme to prevent contamination of amplification products.

Main Components

Components	Specification
CHO qPCR MIX	1.0 mL×2 Tubes
CHO Internal Standard*	1.0 mL × 1 Tube
CHO Negative Control	1.0 mL×2 Tubes
CHO Calibrator ST1	0.5 mL × 1 Tube (3 fg/μL)
CHO Calibrator ST2	0.5 mL × 1 Tube (30 fg/μL)
CHO Calibrator ST3	0.5 mL × 1 Tube (300 fg/μL)
CHO Calibrator ST4	0.5 mL × 1 Tube (3 pg/μL)
CHO Calibrator ST5	0.5 mL × 1 Tube (30 pg/μL)
CHO Calibrator ST6	0.5 mL × 1 Tube (300 pg/μL)

Notes

Components in different batch numbers of reagent kits are not interchangeable.

Reagents required for experimental procedures but not included in the kit: nucleic acid extraction or purification kit.

*If the internal standard function in the kit is not used, no internal standard needs to be added during extraction. The internal standard channel may be omitted during program setup, and the internal standard results need not be considered during result analysis.

*If no internal standard is added during sample extraction but is introduced during amplification, 1 μL of internal standard should be added per reaction mixture when preparing the MIX. When aliquoting the qPCR MIX, use 21 μL per reaction, resulting in a total PCR reaction volume of 31 μL per reaction.

Storage conditions and shelf life

1. Store in the dark at ≤ -20℃ with a validity period of 24 months.
2. Avoid repeated freeze-thaw cycles; the number of repeated freeze-thaw shall not exceed 10 times.
3. The product validity period and expiration date are indicated on the product label.

Applicable Instruments

Including but not limited to the following models: SLAN-96P, SLAN-96S Automatic Medical PCR Analysis System; ABI7500, ABI QuantStudio™ 5 Real-Time Fluorescent Quantitative PCR Instrument; Roche LightCycler 480 Fluorescent Quantitative PCR Instrument; Bio-Rad CFX96 Quantitative PCR Instrument.

Detection method

Take out CHO qPCR MIX, CHO Internal Standard, CHO Negative Control and CHO Calibrators ST1-ST6 from the kit, thaw at room temperature, fully vortex and mix well, then centrifuge briefly for later use.

1. Preparation of Extraction and Recovery Control (ERC)

Prepare the spiked concentration of CHO DNA in ERC as needed (taking the preparation of ERC with 3pg CHO DNA spiked as an example), the specific operations are as follows:

1)Add 100 μL of the sample into a clean 1.5 mL centrifuge tube;
2) Add 10 μL of ST3, mix well and centrifuge briefly, and label it as sample ERC. Perform nucleic acid extraction and purification on the sample ERC together with the test samples of the same batch to obtain sample ERC nucleic acid.
2. Preparation of Reaction Mixture
2.1 Calculate the required number of PCR reaction solutions according to the number of test samples, and it is generally recommended to set 3 replicates per sample.
Number of PCR reaction solutions = (6 concentration gradients of standard curve + 1 No Template Control (NTC) + 1 Negative Control Sample (NCS) + test samples + sample ERC) × 3. Then dispense the corresponding number of PCR reaction solutions into a 96-well PCR plate or PCR eight-tube strips at 20 μL per well.
2.2 Example of sample addition for each reaction well:

Component	Sample Volume
Standard Curve	20 μL CHO qPCR MIX + 10 μL ST1/ST2/ST3/ST4/ST5/ST6
NTC	20 μL CHO qPCR MIX + 10 μL Negative Control
NCS	20 μL CHO qPCR MIX + 10 μL NCS Purified Solution
Test Sample	20 μL CHO qPCR MIX + 10 μL Test Sample Purified Solution
Sample ERC	20 μL CHO qPCR MIX + 10 μL Sample ERC Purified Solution

3.Nucleic Acid Extraction of Samples
Follow the instruction manual of the nucleic acid extraction or purification kit for the operation steps, with a sample volume of 100μL and an internal standard addition volume of 10μL.
4.Fluorescent PCR Reaction
4.1 Add the nucleic acid to the CHO qPCR MIX according to the method in 2.2, cover the reaction tube caps or seal the 96-well PCR plate with an optical film, mix well, centrifuge briefly, and transfer to the fluorescent PCR instrument.
4.2 Run the following program on the fluorescent PCR instrument:

Steps	Conditions	Cycles
UDG Treatment	50°C: 2 minutes	1
Pre-denaturation	95°C: 3 minutes	1
PCR Amplification	95°C: 10 seconds, 60°C (fluorescence collection): 30 seconds	45

Select FAM and CY5 as the fluorescence channels, where FAM is for CHO DNA and CY5 is for the internal standard. For ABI series instruments, select ROX as the reference fluorescence.
5.Result Determination
5.1 Threshold Setting
Adjust the threshold according to the instrument noise level. The threshold can be set as the mean fluorescence intensity of the 3rd to 15th cycles plus 10 times the standard deviation, or take the highest fluorescence value of the negative control as the fluorescence threshold. Set the threshold line to a position that exceeds the fluctuation of the background signal.
5.2 Interpretation of Internal Standard
For negative results, the Ct value of the internal standard should be ≤35; for positive results, the internal standard may have no value or poor value due to competitive inhibition.
5.3 Test Validity Criteria
1) NTC and NCS samples showed no Ct value or their Ct values were higher than the Ct mean of the calibrator's lowest concentration;
2) The linear correlation coefficient R^2 of the standard curve should be ≥0.98, with the slope ranging from-3.1 to-3.8;
3) The recovery rate of spiked samples in each group (if applicable) should be between 50% and 150%.
6.Result Analysis
6.1 Taking SLAN-96P as an Example:
1) If the threshold needs to be adjusted, set the threshold to an appropriate level in the "Parameter Settings" of the "Experiment Analysis" panel;
2) In the "Plate Editing" panel, set the "Sample Type" of the calibrators to "Standard", and assign the values 300000, 30000, 3000, 300, 30, 3 (referring to the DNA concentration added to each well, unit: fg/μL) in the "Attribute" column respectively, and name them ST6, ST5, ST4, ST3, ST2, ST1 in the corresponding "Sample Name" column;

3) In the "Standard Curve" panel of "Experiment Analysis", the slope, intercept, correlation coefficient and amplification efficiency of the standard curve can be read directly;
4) In the "Reaction Well Information Table" panel of "Experiment Analysis", the concentration value of the test result can be read in the "Concentration" column (unit: fg/μL). The unit can be converted to pg/μL or pg/mL in the test report according to user requirements. Calculate the spiked recovery rate based on the test results of the test sample and sample ERC, which is required to be between 50% and 150%.
6.2 Taking ABI 7500 qPCR Instrument (Software Version 2.4) as an Example:
1) If the threshold needs to be adjusted, set the Threshold of FAM channel and CY5 channel to an appropriate position in the Amplification Plot panel of the Analysis module;
2) In the Plate Setup panel of the Setup module, set the Task of the standard curve wells to "Standard", and assign the values 300000, 30000, 3000, 300, 30, 3 (referring to the DNA concentration added to each well, unit: fg/μL) in the Quantity column respectively, and name them ST6, ST5, ST4, ST3, ST2, ST1 in the corresponding Sample column. Set the Task of the NTC well to "NTC", set the Task of NCS wells, test sample wells and sample ERC wells to "Unknown", and name them NTC, NCS, S, ERC in the corresponding Sample Name column, then click "Analyze";
3) In the Standard Curve panel of the Analysis module, the Slope, Y-Inter, R^2 and other parameters of the standard curve can be read directly;
4) In the View Well Table panel of the Analysis module, the concentration value of the test result can be read in the Quantity column (unit: fg/μL). Calculate the spiked recovery rate based on the test results of the test sample and sample ERC, which is required to be between 50% and 150%.

Precautions

1. Store the kit at -20℃ or below.
2. Read the instruction manual of this kit carefully before the experiment and strictly follow the operation steps. Precise control of reaction time, reagent volume and other parameters during operation can ensure optimal test results.
3. Ensure that all consumables used for nucleic acid extraction are clean and free of DNase/RNase. Perform the extraction process as quickly as possible, and proceed to the next experimental step or freeze the extracted nucleic acid for storage immediately after completion.
4. Do not use expired kit components or mix components from different batch numbers.
5. Thaw frozen reagents completely at room temperature before use, and centrifuge briefly to make all liquids sink to the bottom of the tube. Avoid repeated freeze-thaw cycles to prevent degradation of reagent performance.
6. Seal the reaction tube caps with new disposable PE gloves; avoid touching the reaction tubes with bare hands or used gloves. Wear disposable powder-free latex gloves without fluorescent substances during the entire detection process..
7. The laboratory shall be managed in separate areas in strict accordance with relevant regulations, and gene detection experiments shall be carried out in the order of Reagent Preparation Area → Template Extraction Area → Amplification Area → Analysis Area. Strict requirements shall be implemented for the flow of personnel, equipment, reagents and air between different areas.
8. Recap samples, calibrators and other reagents immediately after use to avoid false positive results caused by cross-contamination between components or aerosol contamination.
9. Do not open the caps of tubes containing amplification products. Collect all experimental waste in a timely manner and perform harmless treatment in an area far from the PCR laboratory.
10. If the sample is strongly positive, the detection of the internal standard may be affected due to the competitive inhibition of the reaction system.
11. It is recommended to use the latest version of the operating software for each instrument for experiment and data analysis

Disclaimer

In all cases, the company's liability for this product is limited to the product's value itself.