E.coli residual DNA detection kit (qPCR)

BIORI BIOTECH

Principle

This kit adopts the PCR-fluorescent probe method, and designs primers and probes for the conserved sequences of the E.coli genome to realize quantitative detection of E.coli residual DNA in samples. The kit contains an exogenous internal standard that participates in extraction and amplification together with the sample to monitor abnormalities in extraction and amplification and prevent false negatives. It also contains Uracil-DNA Glycosylase (UDG) to prevent contamination from amplification products.

Specification

100 Reactions/kit

Intended Use

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

Main Components

Components	Specification (100 Reactions/kit)
E.coli qPCR MIX	1.0 mL×2 tubes
E.coli Internal Standard*	1.0 mL×1 tube
E.coli Negative Control	1.0 mL×2 tubes
E.coli Calibrator ST1	0.5 mL×1 tube（30 fg/μL）
E.coli Calibrator ST2	0.5 mL×1 tube（300 fg/μL）
E.coli Calibrator ST3	0.5 mL×1 tube（3 pg/μL）
E.coli Calibrator ST4	0.5 mL×1 tube（30 pg/μL）
E.coli Calibrator ST5	0.5 mL×1 tube（300 pg/μL）

Notes：

Components from kits of different lot numbers cannot be interchanged.
Reagents required for the experimental operation but not provided in the kit: Nucleic acid extraction or purification kit.
*If the internal standard function of the kit is not used, no internal standard needs to be added during extraction, the ROX channel is not set in program configuration, and the ROX results do not need to be considered in result analysis.
*If no internal standard is added during sample extraction but added during amplification, add 1 μL of internal standard to each reaction mixture when preparing the MIX, and dispense 21 μL of qPCR MIX per reaction during aliquoting. In this case, the total PCR reaction volume is 41 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 freeze-thaw cycles shall not exceed 10 times.

3、The product validity period and expiration date are indicated on the label.

Applicable Instruments

Including but not limited to the following models: SLAN-96P, SLAN-96S Fully 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.

Test Method

Take E.coli qPCR MIX, E.coli Internal Standard, E.coli Negative Control and E.coli Calibrators ST1-ST5 out of the kit, thaw at room temperature, vortex and mix thoroughly, then perform brief centrifugation for later use.
1. Preparation of Spike Recovery Control (ERC)
Set the spiked concentration of E.coli DNA in ERC as needed (taking the preparation of ERC with 30 pg of E.coli DNA added as an example), the specific operations are as follows:
Add 100 μL of the test sample into a clean 1.5 mL centrifuge tube.
Add 10 μL of ST3, mix thoroughly and perform brief centrifugation, and label it as sample ERC. Extract and purify nucleic

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acids from the sample ERC and the test samples of the same batch together to obtain sample ERC nucleic acid.

2. Preparation of Reaction Mixture

2.1 Calculation of the required volume of PCR reaction mixture

Calculate the number of required PCR reaction mixtures according to the number of test samples; it is generally recommended to run 3 replicates per sample.Number of PCR reaction mixtures = (5 concentration gradients of standard curve + 1 No Template Control (NTC) + 1 Negative Control Sample (NCS) + test samples + sample ERC) × 3. Aliquot the corresponding volume of PCR reaction mixture into a 96-well PCR plate or PCR 8-strip tubes at 20μL per well.

2.2 Example of sample addition for each reaction well

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

3. Nucleic Acid Extraction from Samples

Follow the instructions of the nucleic acid extraction or purification kit for the operation steps; the sample volume is 100 μL, and the addition volume of internal standard is 10 μL.

4. Fluorescent PCR Reaction

4.1 Add the nucleic acid to the E.coli qPCR MIX according to the method in 2.2, cap the reaction tubes or seal the 96 well PCR plate with an optical sealing film, mix thoroughly, perform brief centrifugation, and transfer to a fluorescent PCR instrument.

4.2 Run the following program on the fluorescent PCR instrument:

Step	Conditions	Number of Cycles
UDG Treatment	50℃ for 2 minutes	1
Pre-denaturation	95℃ for 3 minutes	1
PCR Amplification	95℃ for 10 seconds, 60℃ (fluorescence collection) for 30 seconds	45

Select FAM and ROX as the fluorescent channels, where FAM is for E.coli DNA and ROX is for the E.coli internal standard. For ABI series instruments, select none for the reference fluorescence.

5. Result Determination

5.1 Threshold Line Setting

Adjust the threshold line according to the instrument noise level: take the mean fluorescence intensity of the 3rd to 15th cycles plus 10 times the standard deviation, or use the peak fluorescence value of the negative control as the fluorescence threshold, or set the threshold line above the background signal fluctuation range.

5.2 Interpretation of Internal Standard

The Ct value of the internal standard for negative results should be ≤35; for positive results, the internal standard may have no detectable value or poor detection due to competitive inhibition.

5.3 Valid Test Criteria

1)NTC and NCS show no Ct value or the mean Ct value is higher than that of the calibrator with the lowest concentration;

2)The linear correlation coefficient R^2 of the standard curve is ≥0.98, and the slope is in the range of -3.1 to -3.8;

3)The spike recovery of each group of spiked samples (if applicable) is in the range of 50%-150%.

6. Result Analysis

6.1 Taking SLAN-96P as an example:

1)If the threshold line needs to be adjusted, set the threshold to an appropriate level in the Parameter Settings of the Experimental Analysis panel;

2)In the Plate Editing panel, set the Sample Type of the calibrators to Standard, assign the values 300, 30, 3, 0.3, 0.03 (representing the DNA concentration added to each well, unit: pg/μL) in the Attribute column respectively, and name them ST5, ST4, ST3, ST2, ST1 in the corresponding Sample Name column;

3)In the Standard Curve panel of Experimental Analysis, the slope, intercept, correlation coefficient and amplification efficiency of the standard curve can be read;

4)In the Reaction Well Information Table panel of Experimental Analysis, the concentration value of the test result can be

read in the Concentration column (unit: pg/μL). The unit can be converted to fg/μL or pg/mL in the test report as required by the user. Calculate the spike recovery based on the test results of the test sample and sample ERC, which is required to be in the range of 50%-150%.

6.2 Taking ABI 7500 qPCR Instrument (Software Version 2.4) as an example:

1)If the threshold line needs to be adjusted, set the Threshold of FAM and ROX channels to an appropriate level in the Amplification Plot panel of Analysis, and click Analyze;

2)In the Plate Setup panel of Setup, set the Task of the standard curve wells to Standard, assign the values 300, 30, 3, 0.3, 0.03 (representing the DNA concentration added to each well, unit: pg/μL) in the Quantity column respectively, and name them ST5, ST4, ST3, ST2, ST1 in the corresponding Sample column. Set the Task of the NTC well to NTC, and 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;

3)In the Standard Curve panel of Analysis, the Slope, Y-Inter, R^2, etc. of the standard curve can be read;

4)In the View Well Table panel of Analysis, the concentration value of the test result can be read in the Quantity column (unit: pg/μL). The unit can be converted to fg/μL or pg/mL in the test report as required by the user. Calculate the spike recovery based on the test results of the test sample and sample ERC, which is required to be in the range of 50%-150%.

Precautions

1.Store the kit at -20℃ or below.

2.Read the instructions of this kit carefully before the experiment and follow the operating steps strictly. Precise control of time, reagent volume, etc. during the operation can yield the best results.

3.Ensure that the consumables for nucleic acid extraction are clean, DNase/RNase-free, and free of E.coli and related products. Perform the extraction as quickly as possible, and proceed to the next experiment or store the extracted nucleic acid frozen after completion.

4.Do not use expired components or mix components from different lot numbers.

5.Thaw frozen reagents completely at room temperature before use and perform brief centrifugation to make the liquid sink to the bottom of the tube completely. Avoid repeated freeze-thaw cycles to prevent affecting the reagent performance.

6.Seal the reaction tubes 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 detection process.

7.The laboratory shall be managed in separate areas in strict accordance with relevant regulations, and genetic testing shall be carried out in the order of Reagent Preparation Area → Template Extraction Area → Amplification Area → Analysis Area. Strict requirements shall be imposed on the personnel, equipment, reagents and air flow direction between each area.

8.Recap samples, calibrators and other reagents immediately after use to avoid false positives caused by contamination between components and aerosol contamination.

9.Do not open the amplification products. Collect the waste generated in the experiment in a timely manner and perform harmless disposal away from the PCR laboratory.

10.If the target in the sample is strongly positive, the detection of the internal standard may be affected due to competitive inhibition of the reaction system.

11.It is recommended to use the latest version of the software for each instrument for the experiment and data analysis.

Disclaimer

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

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