PerfectStart® E.coli DNA Quantification Kit

Cat. No. DH131
Storage: at -20°C for two years
Version No. Version 1.0

Description

This product is a special kit for quantitative detection of *E. coli* host cell DNA in intermediate, semi-finished and finished products of various biological products, using Taqman probe technology with dUTP/UDG system, which can degrade U-containing ssDNA and dsDNA and eliminate cross-contamination caused by PCR products.

Kit Contents

Name	DH131-01	Component
E.coli qPCR SuperMix (2×)	1 ml	PCR enzymes, dNTPs, magnesium ions, PCR buffer, etc.
4 × *E.coli* Primer Probe Mix	500 μl	*E.coli* DNA primer probe
E.coli Standard S1	100 μl	*E.coli* standard DNA, calibrated to 500 pg/μl E.coli nucleic acid
Standard Diluent	1 ml	
Nuclease-free Water	1 ml	

Note: The ingredients of different batches of products cannot be mixed or interchanged.

Applicable instruments: ABI7500, Bio-RadCFX96, Hongshi 96P real-time PCR instrument, etc.

Detection Method

To avoid contamination, the experiment needs to be performed in separate areas!

1. Sample Processing (in the nucleic acid extraction area)

(1) Prepare the control: 10-fold serial dilution of *E. coli* standard S1 (500 pg/μl) to 50 pg/μl, 5 pg/μl, 500 fg/μl and 50 fg/μl to obtain standards S2, S3, S4, S5. For example, the dilution method is: 10 μl S1+90 μl *E.coli* Standard Diluent. And then vortex and briefly centrifuge to obtain S2, and so on.

(2) Preparation of ERC (Extraction Recovery Control) sample for quality control: Set the amount of *E.coli* in the ERC sample as required (for example, the preparation of the ERC sample with 50 pg *E.coli*), add 10 μl S3 to 100 μl of the test sample. The ERC sample undergoes sample pretreatment and nucleic acid extraction together with the sample.

(3) Negative control: Pipet 100 μl of nuclease-free water (or basic solution of biological products). Perform sample pretreatment with the test sample, and then nucleic acid extraction.

(4) Test samples: Use a commercial extraction kit to extract nucleic acids in the samples. The extracted nucleic acid should be detected immediately, **otherwise, it should be frozen at -20±5°C for less than 6 months of storage.**

2. Reagent Preparation

(1) Take out the components in the kit and self-prepared reagents. Place them at room temperature. Wait for the temperature to equilibrate to room temperature. And mix well for later use;

(2) qPCR working solution preparation (avoid direct light during the whole process) Prepare the reaction mix according to the following table and the number of test samples. It is recommended to set a blank control, no template control and ERC sample for each test.

When the test sample is n, the number of reaction mixes to be prepared is N = [Number of test samples(n) × 2 + standard dilutions (5) + negative control (1) + no template control (1)] × 3 + 1.

Note: The test samples × 2 is because it is recommended that each test sample should be subjected to the ERC sample at the same time.

Component	Volume of Component	Concentration
E.coli qPCR SuperMix (2×)	10 μl×N	1×
4×*E.coli* Primer Probe Mix	5 μl×N	1×

(3) Mix the prepared qPCR working solution thoroughly and centrifuge briefly before use.
(4) When the working solution in (3) is ready to use, dispense an equal volume of 15 µl into each microcentrifuge tube (eg, 8-strip tube) and transfer it to the sample processing area.

3. Sample Loading (in the sample processing area)

Add 5 µl of the extracted test sample, ERC sample, negative control and standard dilutions to each PCR tube, cover the tube and centrifuge briefly to make the liquid attach to the bottom of the tube.

4. qPCR Amplification (in the amplification and analysis area)

(1) Fluorescence Channel Selection (As for the ABI qPCR instrument)
Selects the FAM channel to detect *E.coli.* Quantitative Standard needs to set Quantitaty concentration. Standard dilutions S1-S5 are set at values of 500, 50, 5, 0.5, 0.05 (pg/µl). It also needs to set Passive Reference to none. For other instruments, refer to the instrument manual.
(2) qPCR Amplification Program Settings
Set the reaction volume to 20 µl

Temperature	Reaction Time	Cycle	Signal Collection
95℃	30 s	1	
95℃	5 s	40	
60℃	35 s		√

5. Result Analysis

In the result analysis software, set the sample type of the corresponding reaction well as NTC (negative control), Unknown (test sample) and Standard (standard curve sample). Set the concentration of Standard dilutions as 500, 50, 5, 0.5, 0.05 (pg/µl).
After the setup is complete, run the analysis, and the software will automatically generate the standard curve, amplification curve and corresponding values. The correlation coefficient R2 of the generated standard curve should be no less than 0.99, and the slope should be between -3.1 to -3.6 (representing amplification efficiency between 90% and 110%). The Ct value of NTC (negative control) should be no value or ≥40. After the concentration of the test sample is automatically generated according to the standard curve, the concentration of *E.coli* DNA in the original sample is converted.
When the standard curve needs to be adjusted, please refer to the following principles:

- The raw Ct values of DNA Standards (S1-S5) can be filtered according to the principle that the difference of Ct values between duplicate wells is ≤0.3.
- Confirm the effective Ct value range of the standard curve based on the Ct value of the NTC negative control. Ensure that the Δ Ct value between adjacent groups of standards is between 3.1-3.6, and the Ct value of NTC is higher than the standard at the lowest concentration by at least 3.
- If Ct (NTC) > Ct (S5) +3, then the maximum valid Ct value is Ct (S5) and a standard curve should be established using the Ct values generated by DNA Standard S1-S5.
- If Ct (S5) + 3 > Ct (NTC), and Ct (S5) - Ct (S4) is between 3.1-3.6, you can discard S5, use DNA Standard S1-S4 to establish a standard curve, and evaluate the standard curve as required the quality of.
- If Ct (S4) + 3 > Ct (NTC), it indicates that the quantitative system is seriously contaminated, and the experiment needs to be repeated after replacing all components in the system.
- To ensure accurate quantification, use at least 4 Ct (DNA Standards) to create a standard curve.
- If the standard curve parameters are poor and outside the valid range, it is recommended to re-quantify.

6. Quality Control Samples

During the test, to ensure the reliability of the experimental results, it is recommended to add ERC samples and negative quality control NCS samples can be increased for simultaneous nucleic acid extraction and detection steps can be performed.
The two quality control evaluation standards are as follows:

- The extraction-recovery rate is calculated according to the test results of the test sample and the ERC sample. It is required to be between 50% and 150%.
- The Ct value of NCS should be greater than the Ct value of the standard at the lowest concentration.

Limitations of Test Methods

Inappropriate sample collection, transportation and storage conditions, or improper experimental operation and environment are likely to cause false-negative or false-positive results.

Product Performance Index

Refer to the product performance report for details.

Notes

- This product is only used for scientific research use. Please read this manual carefully before use.
- Before the experiment, please acquaint and master the operation methods and precautions of various instruments to be used, and conduct quality control of each experiment.
- Laboratory management must strictly follow the management specifications of PCR gene amplification laboratories. The experimental personnel must undergo professional training. The experimental process is strictly carried out in separate areas. All consumables are only for one-time use. Supplies in different areas and stages cannot be used interchangeably. All testing samples are regarded as infectious substances. During the experiment, wear work clothes and change gloves frequently to avoid cross-contamination between samples. Sample handling and waste disposal should comply with local relevant regulations: "General Guidelines for Biosafety of Microbiological Biomedical Laboratories" and "Medical Waste Management Regulations"
- All reagents must be thawed and mixed thoroughly before use.

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