PerfectStart® Plasmid DNA Quantification Kit

Please read the manual carefully before use

Cat. No. DH171

Version No.Version 1.0

Storage: at -20°C for two years

Description

This product is designed to target conserved sequences across various plasmids, enabling quantitative detection of residual plasmid DNA in a wide range of biological products by probe-based qPCR. It can be used together with magnetic bead-based host cell residual DNA extraction kit (Cat. No.: EH201). In addition, a dUTP/UDG system is introduced into this reaction mix, which can degrade U-containing ssDNA and dsDNA before reverse transcription, eliminating carry-over contamination caused by PCR products.

Features

• High specificity, high sensitivity, high amplification efficiency, and broad sample compatibility.

• Specially optimized qPCR reaction buffer provides faster extension speed, higher sensitivity and specificity.

• Incorporates UDG enzyme and dUTP to effectively prevent carry-over contamination from PCR products, ensuring accurate data.

Kit Contents

Component	DH171-01	Main Ingredients
2×Plasmid qPCR SuperMix	2×750 µl	PCR enzyme, dNTPs, Mg^{2+}, PCR buffer, etc.
6×Plasmid Primer & Probe Mix	500 µl	Plasmid DNA primer & probe
Linear Plasmid DNA Standard (2×10^8 copies/µl)	50 µl	
Circular Plasmid DNA Standard (2.5×10^8 copies/µl)	50 µl	
DNA Diluent	3×1 ml	
Nuclease-free Water	1 ml	

Applicable instruments:ABI series, Bio-Rad CFX series, Bioer LineGene 9600 Plus, etc.

Detection Method

1. Standard Curve Sample Preparation (in the sample processing area)

(1) Take out the Plasmid DNA Standard and DNA Diluent in the kit, put them on ice until they are completely thawed. Then gently shake to mix well and briefly centrifuge. Select either the Linear or Circular Plasmid DNA Standard for standard curve preparation based on the structural characteristics of the samples.

(2) Serial dilution: Take 7 clean 1.5 ml centrifuge tubes, labeled with S0, S1, S2, S3, S4, S5, S6, and add 90 µl of DNA Diluent to each tube. Add 10 µl from Plasmid DNA Standard to S0, mix by shaking and centrifuge briefly. Then take 10 µl from S0 to S1, and so on. Repeat the above steps for serial dilution. Standards S0-S6 concentrations are as follows:

Dilution tube	S0	S1	S2	S3	S4	S5	S6
Linear standard concentration(copies/µl)	2×10^7	2×10^6	2×10^5	2×10^4	2×10^3	2×10^2	2×10^1
Circular standard concentration(copies/µl)	2.5×10^7	2.5×10^6	2.5×10^5	2.5×10^4	2.5×10^3	2.5×10^2	/

(3) The diluted S1-S6 are used as standard curve samples and should be placed on ice for later use, and keep S0 in storage. After the experiment, the remaining standards and diluent are recommended to be stored at -20°C, and remaining standards S1-S6 are recommended to be used within one week.

2. Reagent Preparation (in the reagent preparation area)

(1) Bring the components in the kit and self-prepared reagents to room temperature. And mix well for later use.

(2) qPCR working solution preparation (avoid direct light during the whole process)

Prepare the reaction solution according to the following table and the number of test samples. It is recommended to set a negative control for each test. When the number of test samples is n, the number of reaction mixes to be prepared is N = [Number of test samples (n) + standard curve samples (5 or 6) + negative control NTC (1)] × number of replicate wells + 1.

Component	Volume	Working Concentration
2×Plasmid qPCR SuperMix	15 μl×N	1×
6×Plasmid Primer & Probe Mix	5 μl×N	1×

(3) Mix the prepared qPCR working solution thoroughly and centrifuge briefly before use.

3. Sample Loading (in the sample processing area)

Add 20 μl of the qPCR working solution prepared in the previous step to each centrifuge tube, and add 10 μl of templates to the corresponding wells in sequence: negative control (NTC), test samples, standard curve samples. It is recommended that the above three types of samples should be placed in different zones during the design and layout of the reaction wells to avoid cross contamination and inaccurate test results. After capping the tube or sealing it with an optical film, gently shake to mix well, and centrifuge to make all the liquid attach to the bottom of the tube.

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

Put the PCR reaction tubes into the sample tank of the amplification instrument, set the negative control, test samples, standard curve samples in the corresponding order, and set the sample name.

(1) Fluorescence channel selection

Select the FAM channel (Reporter: FAM, Quencher: none) to detect plasmid DNA;

Set Passive Reference to none.

Set the reaction volume to 30 μl.

(2) qPCR amplification program setup

Temperature	Time	Cycle	Fluorescence signal acquisition
95℃	10 min	1	
95℃	15 s	40	
60℃	1 min		√

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). Input the concentration values for the Standards.After the setup is completed, run the analysis, and the software will automatically generate the standard curve, amplification curve and corresponding values. The correlation coefficient R^2 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%). After the concentration of the sample to be tested is automatically generated according to the standard curve, the concentration of plasimid 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 can be filtered according to the principle that the difference of Ct values between duplicate wells is ≤0.3.

• The NTC should have a higher Ct value than the standard at the lowest concentration, or show no detectable signal.

• Parameter settings for result analysis depend on the specific instrument model and software version, and are generally automatically determined by the system. However, if the threshold line set by the system is too close to the baseline, resulting in significant discrepancies in Ct values between replicates, the threshold line may be manually adjusted to an appropriate position. At this point, the amplification curve morphology can be preliminarily checked for normality before proceeding with data analysis.

• To ensure accurate quantification, at least five concentration points must be used to establish the standard curve.

• If the parameters of the standard curve are suboptimal and fall outside the valid range, it is necessary to conduct the experiment again.

6. Quality Control Samples

During the test, to ensure the reliability of the experimental results, it is recommended to add extraction recovery control (ERC) sample and negative control sample (NCS) for simultaneous nucleic acid extraction and detection steps.

The suggested sample preparation method is as follows:

• ERC sample: Add 20 µl S3 to 180 µl of the sample to be tested, and mix well for ERC;
• NCS sample: 200 µl standard diluent (or biological basic solution) as NCS.
The two quality control evaluation standards are as follows:
• The extraction-recovery rate is calculated according to the test results of the test samples 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 handling, 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. Dedicated instruments and equipment should be used for each stage of the experimental operation, and supplies from different areas or 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.

For research use only, not for clinical diagnosis.

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