# *PerfectStart*® Vero DNA Quantification Kit

Please read the manual carefully before use.
Cat. No. DH111
Version No. Version 1.0
Storage: at -20°C for two years

## Description

This product is used for quantitative detection of Vero cell DNA remaining in various biological products.
The principle of this product is that the amount of nucleic acid in the sample is determined by the detection of the intensity of fluorescence which is proportional to the amount of amplified product during the amplification process by adding a fluorescent probe (TaqMan or Molecular Beacon, etc.) to the PCR system. Vero qPCR SuperMix (2×) in this product contains PerfectStart Taq hot start enzyme (uses 3 monoclonal antibodies to efficiently bind with Taq DNA Polymerase, effectively blocking DNA polymerase activity and preventing non-specific amplification at low temperature), qPCR reaction buffer, dNTPs, PCR enhancer and stabilizer specially optimized for Vero cell DNA detection. In addition, a dUTP/UDG system is introduced into this reaction mix, which can degrade U-containing ssDNA and dsDNA before reverse transcription, eliminating cross-contamination caused by PCR products.

## Features

- High specificity, high sensitivity, high amplification efficiency enabled by 3 kinds of blocking antibodies, and suitable for a wide range of samples.
- Specially optimized qPCR reaction buffer to provide higher extension speed, sensitivity and specificity.
- The use of UDG enzyme and dUTP can effectively prevent cross-contamination of PCR products to ensure accurate data.

## Kit Contents

| Component | DH111-01 | Component |
|---|---|---|
| Vero qPCR SuperMix (2×) | 2 × 750 µl | PCR enzymes, dNTPs, magnesium ions, PCR buffer, etc. |
| 6 × Vero Primer Probe Mix | 500 µl | Vero DNA primer probe |
| Vero DNA Standard S0 | 500 µl | Vero standard DNA |
| Standard Diluent | 3 × 1 ml | |
| Nuclease-free Water | 1 ml | |

Applicable instruments: ABI7500, 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 Vero DNA Standard S0 and Standard Diluent in the kit, put them on ice until they are completely thawed. Then gently shake to mix well and briefly centrifuge;
(2) Serial Dilution: Take 6 clean 1.5ml microcentrifuge tubes, labeled with S1, S2, S3, S4, S5, S6, and add 90 µl of Standard Diluent to each tube. Add 10 µl from S0 to S1, mix by shaking and centrifuge briefly. Then take 10 µl from S1 to S2, and so on. Repeat the above steps for serial dilution. Standards S0-S6 concentrations are as follows:

| Standard | S0 | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|---|
| Concentration | 3 ng/µl | 300 pg/µl | 30 pg/µl | 3 pg/µl | 300 fg/µl | 30 fg/µl | 3 fg/µl |

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

### 2. Reagent Preparation (in the reagent preparation area)

(1) Take out the components and self-prepared reagents in the kit. 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 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 (6) + negative control NTC (1)] × number of replicate wells + 1.

| Component | Volume of Component | Concentration |
|---|---|---|
| Vero qPCR SuperMix (2×) | 15 µl×N | 1× |
| 6 × Vero 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 PCR tube, and add 10 µl of templates to the corresponding wells in sequence: negative control NTC, test samples, standard curve samples (S1-S6). 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 Vero DNA;

Set Passive Reference to none.

Set the reaction volume to 30 µl.

(2) qPCR Amplification Program Settings

| Temperature | Reaction Time | Cycle | Signal Collection |
|---|---|---|---|
| 95°C | 5 min | 1 | |
| 95°C | 5 s | 40 | |
| 60°C | 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 300, 30, 3, 0.3, 0.03 (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 ≥36. After the concentration of the sample to be tested is automatically generated according to the standard curve, the concentration of Vero 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 (S6-S1) 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 ΔCt 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 (S6) +3, then the maximum valid Ct value is Ct (S6) and a standard curve should be established using the Ct values generated by DNA Standard S1-S6.

· If Ct (S6) + 3 > Ct (NTC), and Ct (S6) - Ct (S5) is between 3.1-3.6, you can discard S6. Use DNA Standard S1-S5 to establish a standard curve, and evaluate the quality of standard curve as required

· If Ct (S5) + 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 for simultaneous nucleic acid extraction and detection steps.

The suggested sample preparation method is as follows:

- Preparation of sample loading and recovery quality control ERC: Add 20 μl S3 to 180 μl of the sample to be tested, and mix well for ERC;
- Negative control NCS sample: 200 μl DNA 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 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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