

## Instructions for Use of Plasmid Residual DNA Detection Kit (qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

**Cat. No. HG-ZL001**

### Introduction

Plasmid Residual DNA Detection Kit can quantitatively detect various plasmid DNA residues in samples (such as lentivirus, adenovirus, mRNA, etc.) by analyzing the consensus sequence of plasmids used in the market, such as the replicon from ColE1/pMB1/pBR322/pUC. This kit is based on the principle of TaqMan fluorescence probe, with potent specificity, high sensitivity and reliable performance, and is used as a kit specially designed for the detection of plasmid DNA residues in intermediates, bulk products and final products of various biological products. The kit is equipped with the sample preprocessing kit of our company (Cat. No.: HG-CL100) for sample preprocessing.

The kit is equipped with plasmid quantitative reference (the traceability of reference standard has been completed).

The detection range is from  $4 \times 10^1$  copies/ $\mu$ L to  $4 \times 10^6$  copies/ $\mu$ L.

Formula: Plasmid copy number (copies/ $\mu$ L) =  $6.02 \times 10^{14} \times$  Plasmid concentration (ng/ $\mu$ L)/(Plasmid base number  $\times 660$ )

### Specification

100 Reactions

### Kit components

Table 1: Kit components and storage conditions

Components	Specification	Storage temperature
2× qPCR Reaction Buffer	1.6 mL× 1vial	-20°C
Plasmid Primer&Probe MIX	550 $\mu$ L×1vial	
Quantitative reference 1 ( $4 \times 10^6$ )	300 $\mu$ L× 1vial	
Quantitative reference 2 ( $4 \times 10^5$ )	300 $\mu$ L× 1vial	
Quantitative reference 3 ( $4 \times 10^4$ )	300 $\mu$ L× 1vial	
Quantitative reference 4 ( $4 \times 10^3$ )	300 $\mu$ L× 1vial	
Quantitative reference 5 ( $4 \times 10^2$ )	300 $\mu$ L× 1vial	
Quantitative reference 6 ( $4 \times 10^1$ )	300 $\mu$ L× 1vial	
DNA diluent	1.5mL× 3vial	

### Product storage conditions and shelf life

See the above table for storage conditions, and the shelf life is 12 months.

### Applicable model

Including but not limited to ABI7500, BioRad CFX96, Bioer FQD-96A, Roche Light Cycler 480 and other real-time quantitative fluorescence PCR instruments.

## Consumables and equipment to be self-prepared

Please prepare the following consumables and equipment before the test

- ◆ 1.5 mL or 2 mL sterile low adsorption centrifuge tubes
- ◆ Centrifuge
- ◆ 96-well qPCR plate or 8-strip tube adapted to PCR instrument
- ◆ Oscillator
- ◆ 1000  $\mu$ L, 200  $\mu$ L, and 10  $\mu$ L sterile low adsorption pipette tips with cartridge
- ◆ Magnetic stand
- ◆ Fluorescence quantitative PCR instrument
- ◆ Water bath/metal bath
- ◆ Pipettes of various specifications (e.g., 1000  $\mu$ L, 200  $\mu$ L, 10  $\mu$ L, 2.5  $\mu$ L)

## Test steps

### I. Sample Preprocessing

Please refer to the operating instructions of our sample preprocessing kit (Cat. No.: HG-CL100) for details.

### II. qPCR Operation Steps

#### 1. Preparation of quantitative reference and NTC

##### 1.1 Quantitative reference: Ready-to-use

##### 1.2 Preparation of NTC: 100 $\mu$ L DNA diluent

##### 1.3 Preparation of NCS: Take 100 $\mu$ L of DNA diluent and sample for sample preprocessing

##### 1.4 Spike recovery of ERC: It is suggested that 90 $\mu$ L sample + 10 $\mu$ L Quantitative Reference 3 can be prepared in other ways according to the actual situation

#### 2. Preparation and addition of qPCR reaction solution

##### 2.1 Calculate the required number of reaction wells based on the numbers of standards and samples to be tested (generally, 3 replicate wells will be required for each sample):

Number of reaction wells = (quantitative references 1 ~ 6 + 1 negative control (NTC) + test sample)  $\times$  3

##### 2.2 Calculate the total amount of plasmid qPCR MIX required for this time based on the number of reaction wells:

Plasmid qPCR MIX = (Number of reaction wells + 2 or 3)  $\times$  20  $\mu$ L (2 or 3 is operational loss)

##### 2.3 Thaw the reagents to be used on ice, mix by gentle shaking, and prepare the plasmid qPCR MIX as shown in Table 2.

Table 2 Plasmid qPCR MIX Preparation

Components	Volume required for single reaction( $\mu$ L)
2 $\times$ qPCR Reaction MIX	15
Plasmid Primer&Probe MIX	5
Total volume	20

3. Thaw the required reagents on ice, mix well by gentle shaking, and load as shown in Table 3 (total volume of 30  $\mu$ L):

Table 3. Examples of loading to each reaction well

<b>Standards</b>	10 $\mu$ L each of quantitative references 1 ~ 6 + 20 $\mu$ L plasmid qPCR MIX
<b>Negative control (NTC)</b>	10 $\mu$ L each of DNA dilutions + 20 $\mu$ L plasmid qPCR MIX
<b>Test sample</b>	10 $\mu$ L each of test sample + 20 $\mu$ L plasmid qPCR MIX

4. In the experiment, sterile nuclease-free 8-tube strips or 96-well plates should be used for qPCR experiment, bubbles should be removed from the reaction system, and the liquid should be centrifuged to the bottom of the tube to prepare for the reaction.

5. Layout illustration of reaction wells

Table 4. Layout illustration of Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST1	ST1	ST1							S1	S1	S1
B	ST2	ST2	ST2							S2	S2	S2
C	ST3	ST3	ST3							S3	S3	S3
D	ST4	ST4	ST4									
E	ST5	ST5	ST5									
F	ST6	ST6	ST6							ERC-S1	ERC-S1	ERC-S1
G				NTC	NTC	NTC				ERC-S2	ERC-S2	ERC-S2
H				NCS	NCS	NCS				ERC-S3	ERC-S3	ERC-S3

### III. qPCR reaction program and parameter setting

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Create the experimental reaction plate, click Select Fluorophores to select the fluorescence FAM; in the reaction plate diagram, select the Sample well, pull down in Sample Type to select Unknown, check the fluorescence FAM, Target Name is designated as ZL; input the number of replicates for each sample and Sample Name.

2. In the reaction plate diagram, select the Standard well, pull down in Sample Type to select Standard, check the fluorescence FAM, and Target Name is designated as ZL; input the number of replicates for each dilution gradient and Sample Name. And the Concentration column of STD1, STD2, STD3, STD4, STD5, and STD6 is assigned values of 4.00E+06, 4.00E+05, 4.00E+04, 4.00E+03, 4.00E+02, 4.00E+01 (in copies/ $\mu$ L), respectively.

Click "Start Run" on the "Run" interface to perform PCR analysis.

Table 5. PCR reaction program

Stage1	Contamination digestion	Reps: 1	50°C	2 min
Stage2	Pre-denaturation	Reps: 1	95°C	1min
Stage3	Cyclic reaction	Reps: 40	95°C	10 s
			60°C	30 s

Note: The reaction volume is 30  $\mu$ L. Set the program at 60°C for 30 s for fluorescence collection; The collection time in the fluorescence collection step of some instruments is not allowed to be set to 30 s or shorter. For ABI 7000, ABI 7300 and ABI 7500, it can be changed to 35 s. For other equipment, please consult the relevant manufacturer.

## IV. qPCR result analysis

Taking the CFX96 qPCR system (BIO-RAD) as an example.

1. Click Quantitation in Data Analysis Window to read the slope, intercept, amplification efficiency (Effect) and R<sup>2</sup> of the standard curve.
2. In the window Quantitation Data, the SQ Mean column reads the RCL test values of the no-template control (NTC) and the test sample in copies/μL.
3. Data reliability evaluation:

- The difference in Ct values between 3 replicate wells shall be less than 1.0, except for wells with Ct value greater than 35;
- The CT values of negative controls NTC and NCS should be greater than the CT value of the lowest concentration of the standard curve, or the criteria should be set based on the laboratory's own validation results;
- Linear correlation coefficient R<sup>2</sup> of the standard curve shall be equal or greater than 0.98, amplification efficiency shall be within 85%-110%;
- The recovery of ERC shall be within 50%-150% (spike recovery = ERC/(0.9\*sample + 0.1\*Quantitative Reference 3)

## Precautions

1. The kit has been validated for the stability (freezing-thawing and other factors) and does not require dispensing.
2. The preparation for negative samples and positive samples (reference and samples to be tested, etc.) should be separated into different environments and should not be operated in one area. The preparation personnel should wear neat masks, gloves and cleanroom garment.
3. Tips shall be changed between different loading steps in time to avoid cross-contamination and long-time opening.
4. The kit must be used within the shelf life.
5. All components in the kit are recommended to be used after melting in a low temperature environment.
6. The best detection effect can be ensured only by strictly following the instructions and using all the reagents provided with this kit
7. Subsequent qPCR detection shall be performed immediately after sample preprocessing and purification as far as possible on the same day to ensure the accuracy of test results.
8. The final test results are closely related to the efficacy of reagents, operator's operation methods and test environment.
9. Our company is only responsible for the kit itself and not for the sample consumption caused by the use of the kit. Users should fully consider the possible usage of sample before use and reserve sufficient samples.
10. This kit is for in vitro research use only and is not used for clinical diagnosis.。

## Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

