Instructions for Use of Vero Residual DNA Detection Kit (qPCR)

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

Cat. No. HG-VE001

Introduction

The Vero Residual DNA Detection Kit is a kit specially designed for the quantitative detection of residual DNA of Vero host cells in intermediates, bulk and final products of various biological products. This kit can quantitatively detect residual Vero DNA in samples based on the TaqMan fluorescence probe principle. The kit is a rapid, specific and reliable device, with the minimum detection limit reaching fg level.

The supporting Vero DNA quantitative reference provided in the kit has been traced to the national standard.

The detection range of this kit is: 3 fg/μL ~ 3 × 10^5 fg/μL.

Specification

100 Reactions

Kit components

Table 1: Kit components and storage conditions

Fill volume of component	Filling volume	Storage conditions
2×qPCR Reaction MIX	1.6 mL × 1 vial	-20℃ or below, protected from light
Vero Primer&Probe MIX	550 μL × 1 vial	
Quantitative standard 1 (300 pg/μL)	300 μL × 1 vial	
Quantitative standard 2 (30 pg/μL)	300 μL × 1 vial	
Quantitative standard 3 (3 pg/μL)	300 μL × 1 vial	
Quantitative standard 4 (0.3 pg/μL)	300 μL × 1 vial	
Quantitative standard 5 (0.03 pg/μL)	300 μL × 1 vial	
Quantitative standard 6 (0.003 pg/μL)	300 μL × 1 vial	
DNA diluent	1 mL × 3 vials	

Shelf life

Twelve (12) months at specified storage conditions.

Apparatus to be prepared by the user

Fluorescent quantitative PCR system	1000 μL, 100 μL, 10 μL pipette
1.5 mL sterile centrifuge tube	1000 μL, 100 μL, 10 μL sterile low-retention filtered tips

Sterile, enzyme-free 8-strip PCR tubes or 96-well qPCR plate

Adaptive models (including but not limited to)

- ABI QuantStudio 3 qPCR System
- Roche LC96 Real-Time PCR System
- ABI 7500 Real-Time PCR System
- Bio-Rad CFX Opus96 Real-Time PCR System
- RocGene ArchimedTM X Real-Time PCR System

Test procedures

Preparation and addition of qPCR reaction solution

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 = (6 sets of references + 1 no-template control (NTC) + test sample) × 3

2. Calculate the total amount of Vero qPCR MIX required for this time based on the number of reaction wells:

Vero qPCR MIX = (Number of reaction wells + 2 or 3) × 20 μL (2 or 3 is the amount of operational loss)

3. Thaw the reagents to be used on ice, mix well by gentle shaking, and prepare the Vero qPCR MIX as shown in Table 2.

Table 2 Vero qPCR MIX Preparation

Components	Volume required for single reaction
2×qPCR Reaction MIX	15 μL
Plasmid Primer&Probe MIX	5 μL

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

Table 3. Examples of loading to each reaction well

Template	Volume for the template	Volume of CHO qPCR MIX required
Standards	10 μL each of references 1-6	20 μL
No template control (NTC)	10 μL for each DNA diluent buffer	20 μL
Test sample	10 μL for each test sample	20 μL

5. Sterile nuclease-free eight-tube strips or 96-well plates can be used for the reaction. It is necessary to remove the bubbles in the reaction system and centrifuge the liquid to the bottom for preparing the reaction.

qPCR reaction program and parameter setting

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

1. Set up the reaction program:

2. Create the test 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 Vero; input the number of replicates for each sample and Sample Name.

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 Vero; 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 with values of 300000, 30000, 3000, 300, 30 and 3 (in pg/μL), respectively.

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

Stage1	Contamination digestion	Reps: 1	50℃	2min
Stage2	Pre-denaturation	Reps: 1	95℃	20s
Stage3	Cyclic reaction	Reps: 40	95℃	3s
			60℃	30s

Stage 3 in the program is set as fluorescence collection at 60℃ for 30 s;

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^2 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. For NTC, the result should be N/A, or the Ct value should be greater than the mean Ct value of the lowest concentration on standard curve.

Precautions

1. This kit is for *in vitro* detection only, and may not be used for clinical diagnosis.
2. The kit must be used within the shelf life.
3. All components in the kit should be used after thawing in a low-temperature environment.
4. The optimal assay results may only be achieved by strictly following the instructions and using only the reagents provided in the kit.
5. Please timely replace pipette tips when loading different samples and performing different steps, so as to avoid cross contamination; opening the reagent caps for a long time should also be avoided.
6. The final assay results are closely related to reagent effectiveness, the operations of analysts, and the test environment.
7. Our company is only responsible for the kits themselves, and will not be responsible for the sample consumption caused by kits during use. Users should fully consider the possible sample consumption before operation, and should reserve sufficient sample size.

Disclaimer

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

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