Plasmid DNA Residue (qPCR) Detection Kit - Instruction

Catalog Number：ARK6618

【Introduction to kit】

Plasmid DNA residue (qPCR) detection kit can quantitatively detect the residues of various types of plasmid DNA in samples (such as lentivirus, adenovirus, etc.) by analyzing the common DNA sequence of plasmids used in the market. Based on the TaqMan fluorescence probe principle, this kit has strong specificity, high sensitivity and reliable performance. It is a special kit for the detection of plasmid DNA residues in intermediate, semi-finished and finished products of various biological products.

This kit is equipped with linearized and nonlinear reference products, which can be selected independently according to requirements. The linearized detection range is 4×10^6 copies/μL to 4×10^1 copies/μL. The detection range of nonlinearization is 2×10^6 copies/μL to 2×10^2 copies/μL. This kit can be used together with AREX Biosciences host cell residual DNA sample pretreatment kit, and can accurately quantify the trace plasmid DNA in the sample.

Formula: plasmid copy number (copies/μL)=6.02×10^{14}×plasmid concentration (ng/μL)/(plasmid base number×660)

【Components of the kit】

Table 1 Composition and storage conditions of the kit

Components	Size	Storage Conditions
Plasmid Primer&Probe MIX	550 μL×1 tube	-20℃ (keep in dark)
2×qPCR Reaction MIX	1.6 mL×1 tube	-20℃
Linearized DNA Quantitative Reference (4×10^8copies/μL)	50 μL×1 tube	-20℃
Nonlinear DNA Quantitative Reference (2×10^8copies/μL)	50 μL×1 tube	-20℃
DNA diluent	1.5 mL×3 tubes	-20℃

【Specifications】

100 Reactions

【Period of validity】

Valid for 12 months under the prescribed storage conditions .

【Self provided equipment required for test】

Fluorescence quantitative PCR instrument

1.5mL centrifuge tube with sterile, and low retention

Optical 8-tube strip or 96-well qPCR plate with sterile, DNase/RNase-free and low retention

1000 μL, 100 μL, 10 μL pipette gun

1000 μL, 100 μL, 10 μL pipette tip with sterile, low retention and filter element

【Applicable models (including but not limited to)】

ABI QuantStudio 3 or 5 Real-Time PCR System

ABI 7500 Real-Time PCR System

Bio-Rad CFX Opus96 Real-Time PCR System

【Testing procedures】

- **Dilution of DNA quantitative reference**

The linearized DNA quantitative reference (4×10^8copies/μL) and nonlinear DNA quantitative reference (2×10^8copies/μL) series were diluted with the DNA diluent provided by the kit (dilution: 10 times).Linearized DNA quantitative reference (4×10^8copies/μL) were successively diluted to 4×10^7 copies/μL, 4×10^6 copies/μL, 4×10^5 copies/μL, 4×10^4 copies/μL, 4×10^3 copies/μL, 4×10^2 copies/μL, 4×10^1 copies/μL; The nonlinear DNA quantitative reference (2×10^8 copies/μL) was successively diluted to 2×10^7 copies/μL, 2×10^6 copies/μL, 2×10^5 copies/μL, 2×10^4 copies/μL, 2×10^3 copies/μL, 2×10^2 copies/μL. The specific steps are as follows:

1. The DNA quantitative reference and DNA diluent in the kit were melted on ice or at 2-8°C. After complete melting, slightly oscillate and mix well, quickly centrifuge for 3-5 s in a short time.

2. If use linearized DNA quantitative reference, take seven clean 1.5ml centrifuge tubes and label as ST0, ST1, ST2, ST3, ST4, ST5, and ST6, respectively. If use nonlinear DNA quantitative reference,

take six clean 1.5ml centrifuge tubes and label as ST0, ST1, ST2, ST3, ST4, and ST5, respectively.

3. The DNA quantitative reference was diluted 10 times with DNA diluent in ST0 tube, mixed with slight oscillation, and centrifuged for 3~5 s. Ensure that the DNA quantitative reference is thoroughly mixed with the DNA diluent, and avoid repeated freezing and thawing during use.

4. 90 μL DNA diluent was added to ST1, ST2, ST3, ST4, ST5 and ST6 tubes respectively, and then gradient dilution was performed. The dilution method is shown in Table 2.

Table 2 Dilution of DNA quantitative reference

Dilution tube	Dilution volume	Final concentration (copies/μL)	
		Linearized DNA Quantitative Reference	Nonlinear DNA Quantitative Reference
ST1	10 μL ST0+90 μL DNA diluent	4×10^6	2×10^6
ST2	10 μL ST1+90 μL DNA diluent	4×10^5	2×10^5
ST3	10 μL ST2+90 μL DNA diluent	4×10^4	2×10^4
ST4	10 μL ST3+90 μL DNA diluent	4×10^3	2×10^3
ST5	10 μL ST4+90 μL DNA diluent	4×10^2	2×10^2
ST6	10 μL ST5+90 μL DNA diluent	4×10^1	/

Note :

1. The concentration points of the standard curve can be selected according to the actual verification results, and should contain at least 5 concentration points.

2. Melted and unused DNA diluent can be stored at 2-8°C for a short time. If it is not used for a long time, it should be stored at -20°C.

3. The dilution method can be adjusted according to the experimental needs.

- **Preparation and sample adding of qPCR reaction solution**

1. Calculate the number of holes required for reaction according to the number of DNA quantitative references and samples to be tested (generally 3 groups of multiple holes are made):

Number of reaction holes=(linearized DNA quantitative references ST1 to ST6 and nonlinear DNA quantitative references ST1 to ST5 + NTC+samples to be tested)×3

(Linearized DNA references have 6 concentration gradients and nonlinear DNA references have 5 concentration gradients)

2. Calculate the total amount of qPCR MIX required according to the number of reaction wells: qPCR MIX=(number of reaction wells+2 or 3)×15 μL(2×qPCR Reaction Mix)+(number of reaction wells+2 or 3)×5 μL(Plasmid Primer&Probe mix) (2 or 3 is the operating loss)

3. Put all reagents on ice to melt, shake slightly and mix well, and prepare qPCR MIX as shown in Table 3.

Table 3 Preparation of qPCR Mix

Components	Single reaction amount
2×qPCR Reaction MIX	15 μL
Plasmid Primer and Probe MIX	5 μL
Total volume	20 μL

4. Put the required reagent on ice to melt, shake slightly and mix well, and add samples as shown in Table 4 and Table 5 (total volume 30μL).

Table 4. Sample adding examples of each reaction hole (Linearized DNA Quantitative Reference)

Template	Template amount	qPCR MIX amount
Reference	10 μL of references ST1 to ST6	20 μL
Template-free NTC	10 μL of each DNA diluent	20 μL
Sample to be tested	10 μL of each sample to be tested	20 μL

Table 5 Sample adding examples of each reaction hole (Nonlinear DNA Quantitative Reference)

Template	Template amount	qPCR MIX amount
Reference	10 μL of references ST1 to ST5	20 μL
Template-free NTC	10 μL of each DNA diluent	20 μL
Sample to be tested	10 μL of each sample to be tested	20 μL

5. The test can be performed by using optical 8-tube strip or 96-well qPCR plate. Bubbles in the reaction system should be removed and centrifuged to be bottom of the tube.

- **qPCR reaction procedure and parameter setting**

Take American ABI QuantStudio 3 Real-Time PCR System and software version 1.4.3 as an example.

1. 1Create a blank new program. Experiment type: Standard Curve, Chemistry: TaqMan® Reagents, Run mode: Standard.

2. Create a new detection probe, name it plasmid DNA, select FAM for reporting fluorophores and None for quenching fluorophores (reference fluorescence is ROX).

3. Set the reaction procedure:

Stage1	Pollution to digest	Reps:1	50℃	2 min
Stage2	Predenaturation	Reps:1	95℃	20 s
Stage3	Cyclic reaction	Reps:40	95 ℃ 60 ℃	3 s 31 s

Stage3 is set to fluorescence collection at 60 ℃ for 31 s.

4. Click the“Start Run”button in the Run interface to perform PCR measurement.

- **qPCR result analysis**

Take American ABI QuantStudio 3 Real-Time PCR System and software version 1.4.3 as an example.

1. In the Plate interface, the Task column of the standard curve hole was set as "S", and the linearized DNA standard curves were assigned in Quantity column as 4×10^6, 4×10^5, 4×10^4, 4×10^3, 4×10^2, 4×10^1 (unit: copies/μL), respectively. And in the corresponding Sample name column named ST1, ST2, ST3, ST4, ST5, ST6; The nonlinear DNA standard curves were assigned values of 2×10^6,2×10^5,2×10^4,2×10^3,2×10^2 (unit: copies/μL), and named ST1, ST2, ST3, ST4, and ST5 in the corresponding Sample name column.

2. In the Plate interface, set the Task column of the non-template control NTC hole to "N", set the Task column of the Sample hole to be tested to "U", and name the name of the sample in the corresponding Sample name column.

3. Click Analyze and check whether the Amplification curve is normal in the Amplification Plot panel of the Results.

4. In the Standard Curve panel of Results, the Slope, R^2 and amplification efficiency (Eff%) of the standard curve can be read.

5. In the View panel of Results, Quantity Mean column can read the detected value of Quantity Mean

sample without template control NTC, in copies/μL.

- The parameter setting of the result analysis needs to be based on the specific model and the software version used, and can also be automatically interpreted by the instrument.
- NTC test results should be Undetermined or Ct value >35.

【Precautions】

1. This kit has passed the verification of stability (freeze-thaw and other factors), and does not need to be divided into multiple tubes.
2. The preparation environment of negative reagents and positive reagents should be divided into different areas, and cannot be operated in one area.
3. Pay attention to replacing the tips in time between different sampling steps to avoid cross contamination, and avoid opening the tube cover for a long time.
4. The kit must be used within the validity period.
5. All components in the kit are recommended to be used after melting in a low temperature environment.
6. Only by strictly observing the operating methods of the instructions and using all the reagents matched with this kit can the best detection effect be ensured.
7. The final test result is closely related to the effectiveness of the reagent, the operator's operation method and the test environment.
8. The company is only responsible for the kit itself, and is not responsible for the sample consumption caused by the use of the kit. Please fully consider the possible usage of samples and reserve enough samples before use.
9. This kit is only for in study, not for clinical diagnosis.