E1A Residual DNA Fragment (qPCR) Detection Kit - Instruction

Catalog Number：ARK6648

【Introduction to kit】

E1A residual DNA fragment (qPCR) detection kit is a special kit for quantitative detection of E1A residual DNA fragments in intermediates, semi-finished products and finished products of various biological products. This kit uses TaqMan fluorescence probe principle to quantitatively detect E1A residual DNA fragments in samples. The detection is fast, specific and reliable. The minimum detection limit can reach 4 copies/μL.

【Components of the kit】

Table 1 Composition and storage conditions of the kit

Components	Total amount	Storage Conditions
E1A Primer&Probe MIX-99bp	550 μL×1 tube	-20℃ (keep in dark)
E1A Primer&Probe MIX-165bp	550 μL×1 tube	
E1A Primer&Probe MIX-274bp	550 μL×1 tube	
E1A Primer&Probe MIX-554bp	550 μL×1 tube	
2×qPCR Reaction MIX	1.6 mL×4 tubes	-20℃
Linearized DNA quantitative reference (4×10^8copies/μL)	50 μL×1 tube	
DNA diluent	1.5 mL×3 tubes	

【Specifications】

4×100 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 Real-Time PCR System

LightCycler 480 Real-Time PCR System

ABI 7500 Real-Time PCR System

Bio-Rad CFX Opus96 Real-Time PCR System

Archimed™ X Real-Time PCR System

【Testing procedures】

- **Dilution of DNA quantitative reference**

Gradiently dilute E1A DNA quantitative reference(4×10^8 copies/μL) with DNA diluent provided by the kit, and the dilution concentration is 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, 4×10^0 copies/μL. The specific steps are as follows:

1. The E1A 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, and repeat 3 times.

2. Take eight clean 1.5 mL centrifuge tubes and mark them as ST0, ST1, ST2, ST3, ST4, ST5, ST6 and ST7 respectively.

3. Dilute E1A DNA quantitative reference to 4×10^7 copies/μL with DNA diluent in ST0 tube. The mixture was slightly oscillated, centrifuged for 3-5 s in a short time, and repeated three times to

ensure that the DNA quantitative reference was fully mixed with the DNA diluent, and repeated freeze-thaw was avoided during use.

4. 180 μL DNA diluent was added to ST1, ST2, ST3, ST4, ST5, ST6 and ST7 tubes respectively, and then gradient dilution was performed. The dilution method is shown in Table 2 :

Table 2 Dilution of E1A DNA quantitative reference

Dilution tube	Dilution volume	Final concentration (copies/μL)
ST1	20 μL ST0+180 μL DNA diluent	4×10^{6}
ST2	20 μL ST1+180 μL DNA diluent	4×10^{5}
ST3	20 μL ST2+180 μL DNA diluent	4×10^{4}
ST4	20 μL ST3+180 μL DNA diluent	4×10^{3}
ST5	20 μL ST4+180 μL DNA diluent	4×10^{2}
ST6	20 μL ST5+180 μL DNA diluent	4×10^{1}
ST7	20 μL ST6+180 μL DNA diluent	4×10^{0}

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**

Note: The kit can detect four amplification fragments of different lengths. When establishing a standard curve, and calculate the residual amount of host nucleic acid fragments in the sample based on the standard curve of the corresponding amplified fragment. In order to meet the detection needs of four amplification fragments synchronous, the volume of the sample needs to be ≥120 μL after the sample is extracted. In this regard, it is recommended that each sample to be tested at least 2 tubes for nucleic acid extraction at the same time. After the extraction is completed To be tested.

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

Number of reaction holes=(references ST1 to ST7+ NTC+sample to be tested)×3

2. Calculate the total amount of E1A qPCR MIX required according to the number of reaction

wells: E1A qPCR MIX=(number of reaction wells+2 or 3) × 20 μL (2 or 3 is the operating loss)

Note: The Primer & Probe MX is the E1A Primer & Probe Mix-99/165/274/554bp in the component.

3. Put all reagents on ice to melt, shake slightly and mix well, and prepare E1A qPCR MIX as shown in Table 3-Table 6.

Table 3 Preparation of E1A qPCR Mix-99bp

Components	Single reaction amount
2×qPCR Reaction MIX	15 μL
CHO Primer and Probe MIX-99bp	5 μL

Table 4 Preparation of E1A qPCR Mix-165bp

Components	Single reaction amount
2×qPCR Reaction MIX	15 μL
CHO Primer and Probe MIX-165bp	5 μL

Table 5 Preparation of E1A qPCR Mix-274bp

Components	Single reaction amount
2×qPCR Reaction MIX	15 μL
CHO Primer and Probe MIX-274bp	5 μL

Table 6 Preparation of E1A qPCR Mix-554bp

Components	Single reaction amount
2×qPCR Reaction MIX	15 μL
CHO Primer and Probe MIX-554bp	5 μL

4. Put the required reagent on ice to melt, shake slightly and mix well, and add samples as shown in Table 7-Table 10 (total volume 30 μL).

Table 7 Sample adding examples of each reaction hole

Template	Template amount	E1A qPCR MIX-99bp amount
Reference	10 μL of references ST1 to ST7	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 8 Sample adding examples of each reaction hole

Template	Template amount	E1A qPCR MIX-165bp amount
Reference	10 μL of references ST1 to ST7	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 9 Sample adding examples of each reaction hole

Template	Template amount	E1A qPCR MIX-274bp amount
Reference	10 μL of references ST1 to ST7	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 10 Sample adding examples of each reaction hole

Template	Template amount	E1A qPCR MIX-554bp amount
Reference	10 μL of references ST1 to ST7	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. Create a blank new program. Experiment type: Standard curve, Chemistry: TaqMan® Reagents, Run mode: Standard.

2. Create a new detection probe, named E1A-DNA, with FAM as the reporting fluorescence and None

as the quenching fluorescence(ROX as the reference fluorescence) .

3. Set the reaction procedure

Stage1	Pollution to digest	Reps:1	50℃	2 min
Stage2	Predenaturation	Reps:1	95℃	10 min
Stage3	Cyclic reaction	Reps:40	95℃	15 s
			56℃	30 s
			72℃	1 min

Stage3 is set to fluorescence collection at 72℃ for 1min.

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, set the Task column of standard curve as "S", and assign values to the Quantity column as 4×10^6, 4×10^5, 4×10^4, 4×10^3, 4×10^2, 4×10^1 and 4×10^0 (copies/μL) , and name them ST1, ST2, ST3, ST4, ST5, ST6 and ST7 in the corresponding Sample name column.

2. In the Plate interface, set the Task column of NTC (no template comparison) as "N", set the Task column of samples to be tested as "U", and name the samples in the corresponding Sample name column.

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

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

5. In the View panel of Results, you can read the detection value of NTC and the samples to be tested in the column of Mean Quantity, copies/μL.

- The parameter setting of the analysis results depends on the specific model and the software version used, and it can also be automatically interpreted by the instrument.
- The test result of NTC 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. All components in the kit are recommended to be used after melting in a low temperature environment.

5. 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.

6. The final test result is closely related to the effectiveness of the reagent, the operator's operation method and the test environment.

7. 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.

8. This kit is only for in study, not for clinical diagnosis.