

293T Residual DNA Fragment (qPCR) Detection Kit

Catalog Number: FHT0311C-4×100

【Introduction to Kit】

293T residual DNA fragment (qPCR) detection kit is a special kit for quantitative detection of 293T host cell DNA residual fragments in intermediates, semi-finished products and finished products of various biological products. This kit uses TaqMan fluorescence probe principle to quantitatively detect 293T residual DNA fragments in samples. The detection is fast, specific and reliable. The minimum detection limit can reach fg level.

【Components of the kit】

Table 1 Composition and storage conditions of the kit

Components	Total amount	Storage Conditions
293T Primer&Probe MIX-94bp	550 μ L×1 tube	-20°C (keep in dark)
293T Primer&Probe MIX-153bp	550 μ L×1 tube	
293T Primer&Probe MIX-256bp	550 μ L×1 tube	
293T Primer&Probe MIX-530bp	550 μ L×1 tube	
2×qPCR Reaction MIX	1.6 mL×4 tubes	-20°C
293T DNA Quantitative Reference (30 ng/ μ L)	50 μ L×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 μ L, 100 μ L, 10 μ L pipette gun

1000 μ L, 100 μ L, 10 μ L 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 293T DNA quantitative reference(30 ng/μL) with DNA diluent provided by the kit, and the dilution concentration is 3000 pg/μL, 300 pg/μL, 30 pg/μL, 3 pg/μL, 0.3 pg/μL, 0.03 pg/μL, The specific steps are as follows:

1. The 293T 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 six clean 1.5 mL centrifuge tubes and mark them as ST0, ST1, ST2, ST3, ST4 and ST5 respectively.

3. Dilute 293T DNA quantitative reference to 3000 pg/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.

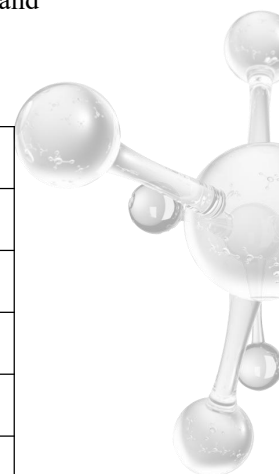
4. 180 μL DNA diluent was added to ST1, ST2, ST3, ST4 and ST5 tubes respectively, and then gradient dilution was performed. The dilution method is shown in Table 2 :

Table 2 Dilution of 293T DNA quantitative reference

Dilution tube	Dilution volume	Final concentration (pg/μL)
ST1	20 μL ST0+180 μL DNA diluent	300
ST2	20 μL ST1+180 μL DNA diluent	30
ST3	20 μL ST2+180 μL DNA diluent	3
ST4	20 μL ST3+180 μL DNA diluent	0.3
ST5	20 μL ST4+180 μL DNA diluent	0.03

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 $\geq 120 \mu\text{L}$ 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 ST5+ NTC+sample to be tested) $\times$ 3

2. Calculate the total amount of 293T qPCR MIX required according to the number of reaction wells: 293T qPCR MIX=(number of reaction wells+2 or 3) $\times$ 20 μL (2 or 3 is the operating loss)

Note: The Primer & Probe MX is the 293T Primer & Probe Mix-94/153/256/530bp in the component.

3. Put all reagents on ice to melt, shake slightly and mix well, and prepare 293T qPCR MIX as shown in Table 3-Table 6.

Table 3 Preparation of 293T qPCR Mix-94bp

Components	Single reaction amount
2 $\times$ qPCR Reaction MIX	15 μL
293T Primer and Probe MIX-94bp	5 μL

Table 4 Preparation of 293T qPCR Mix-153bp

Components	Single reaction amount
2 $\times$ qPCR Reaction MIX	15 μL
293T Primer and Probe MIX-153bp	5 μL

Table 5 Preparation of 293T qPCR Mix-256bp

Components	Single reaction amount
2 $\times$ qPCR Reaction MIX	15 μL
293T Primer and Probe MIX-256bp	5 μL

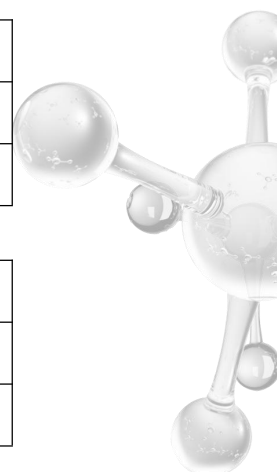


Table 6 Preparation of 293T qPCR Mix-530bp

Components	Single reaction amount
2×qPCR Reaction MIX	15 µL
293T Primer and Probe MIX-530bp	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	293T qPCR MIX-94bp 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

Table 8 Sample adding examples of each reaction hole

Template	Template amount	293T qPCR MIX-153bp 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

Table 9 Sample adding examples of each reaction hole

Template	Template amount	293T qPCR MIX-256bp 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

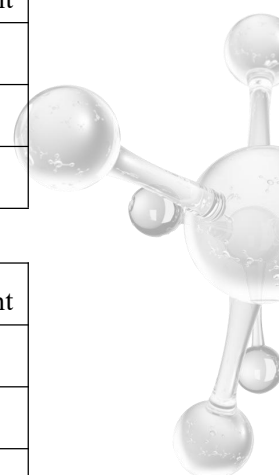
Table 10 Sample adding examples of each reaction hole

Template	Template amount	293T qPCR MIX-530bp 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. Create a blank new program. Experiment type: Standard curve, Chemistry: TaqMan® Reagents, Run mode: Standard.

2. Create a new detection probe, named 293T-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°C	2 min
Stage2	Predenaturation	Reps:1	95°C	10 min
			95°C	15 s
Stage3	Cyclic reaction	Reps:40	56°C	30 s
			72°C	1 min

Stage3 is set to fluorescence collection at 72°C 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 300, 30, 3, 0.3 and 0.03 (pg/μL) , and name them ST1, ST2, ST3, ST4 and ST5 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.

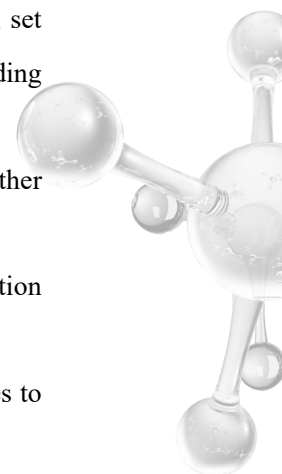
3. Click Analyze, and in the Amplification Plot panel of Results, preliminarily check whether the shape of the amplification curve is normal.

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, pg/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 result of NTC was about 2 to 3 Ct values higher than the lowest point in the standard curve.



【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.

The specification is issued in version A/0 with an effective date of 15th Oct, 2024.

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