Hela Residual DNA (qPCR) Detection Kit

Catalog Number：H0311C-100

【Introduction to Kit】

Hela are widely used for protein expression and rAAV production, and their residual DNA is present as impurities in semi-finished products, intermediates, and finished products. The Hela cell residual DNA detection kit independently developed by JunYan Bio is highly specialized, which can quickly and specifically detect the residual DNA of Hela cells.This kit uses TaqMan fluorescence probe principle to quantitatively detect Hela residual DNA 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
Hela Primer&Probe MIX	550 μL×1 tube	-20℃ (keep in dark)
2×qPCR Reaction MIX	1.6 mL×1 tube	-20℃
Hela DNA Quantitative Reference	50 μL×1 tube	
DNA diluent	1.5 mL×3 tubes	

【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

1000μL, 100μL, 10μL pipette gun

1000μL, 100μL, 10μL 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 Hela 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 Hela 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 seven clean 1.5 mL centrifuge tubes and mark them as ST0, ST1, ST2, ST3, ST4 and ST5 respectively.

3. Dilute Hela 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. 90μL 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 Hela DNA quantitative reference

Dilution tube	Dilution volume	Final concentration (pg/μL)
ST1	10 μL ST0+90 μL DNA diluent	300
ST2	10 μL ST1+90 μL DNA diluent	30
ST3	10 μL ST2+90 μL DNA diluent	3
ST4	10 μL ST3+90 μL DNA diluent	0.3
ST5	10 μL ST4+90 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**

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)×3

2. Calculate the total amount of Hela qPCR MIX required according to the number of reaction wells: Hela qPCR MIX=(number of reaction wells+2 or 3) × 20 μL (2 or 3 is the operating loss)

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

Table 3 Preparation of Hela qPCR Mix

Components	Single reaction amount
2×qPCR Reaction MIX	15 μL
Hela Primer and Probe MIX	5 μL

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

Table4 Sample adding examples of each reaction hole

Template	Template amount	Hela 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. Create a blank new program. Experiment type: Standard curve, Chemistry: TaqMan® Reagents, Run mode: Standard.

2. Create a new detection probe, named Hela-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℃	2min
Stage2	Predenaturation	Reps:1	95℃	10min

Stage3	Cyclic reaction	Reps:40	95℃	3s
			62℃	31s

Stage3 is set to fluorescence collection at 62℃ for 31s.

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 detection was about 2 to 3 Ct values higher than the lowest point in the 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

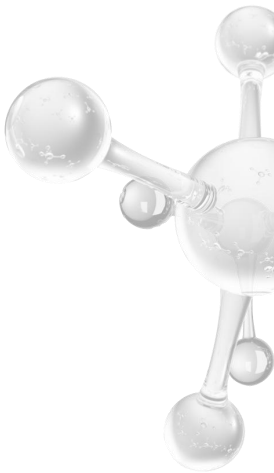
reagents matched with this kit can the best detection effect be ensured.

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 21st Oct,2024.



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