HEK293 Total RNA Residue (qPCR) Detection Kit - Instruction

Catalog Number：ARK6636

【Introduction to kit】

HEK293 Total RNA Residue (qPCR) detection kit is a specialized kit used for quantitative detection of HEK293 total RNA residue in various biological intermediates, semi-finished products, and finished products.This kit uses the TaqMan fluorescent probe principle to quantitatively detect residual RNA of HEK293 in the sample. 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
HEK293 RNA Primer&Probe MIX	1050 μL×1 tube	-20℃ （keep in dark）
HEK293 RNA IPC Primer&Probe MIX	550 μL×1 tube	
One Step qRT-PCR Reaction MIX	420 μL×1 tube	-20℃
One Step Enzyme MIX	105 μL×1 tube	
HEK293 RNA Quantitative Reference (20 ng/μL)	50 μL×1 tube	
RNA Diluent	1.5 mL×3 tubes	

【Specifications】

100 Reactions

【Period of validity】

Valid for 12 months under the prescribed storage conditions .

【Self provided consumables, reagents and equipment required for test】

DNase I（RNase-free）

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 5 Real-Time PCR System

LightCycler 480 Real-Time PCR System

ABI 7500 Real-Time PCR System

【Testing procedures】

- **Dilution of DNA quantitative reference**

Dilute the RNA quantification reference substance (20 ng/μL) with the RNA diluent solution provided by the reagent kit at a ratio of 10 times. The dilution concentrations are 2000 pg/μL,200 pg/μL,20 pg/μL,2 pg/μL,0.2 pg/μL,0.02 pg/μL,and 0.002 pg/μL,respectively. The specific steps are as follows:

1. Melt the RNA quantification reference and RNA diluent in the reagent kit on ice or at 2-8 ℃. After complete dissolution, gently shake and mix well, centrifuge for 3-5 seconds.

2. Take clean 1.5 mL centrifuge tubes and label them as ST0, ST1，ST2，ST3，ST4，ST5，ST6。

3. Dilute the RNA quantification reference substance (20 ng/μL) in an ST0 tube with RNA diluent to 2000 pg/μL, mix well by blowing, and centrifuge instantly. Ensure that the RNA quantification reference material is thoroughly mixed with the RNA diluent solution, and avoid repeated freezing and thawing of the RNA quantification reference material provided by the kit during use.

4. Add 90 μL of RNA diluent solution to ST1, ST2, ST3, ST4, ST5, and ST6 tubes respectively, and then perform gradient dilution. The dilution method is as follows:

Table 2 Dilution of HEK293 RNA quantitative reference

Dilution tube	Dilution volume	Final concentration (pg/μL)
ST1	10 μL ST0+90 μL RNA diluent	200

ST2	10 μL ST1+90 μL RNA diluent	20
ST3	10 μL ST2+90 μL RNA diluent	2
ST4	10 μL ST3+90 μL RNA diluent	0.2
ST5	10 μL ST4+90 μL RNA diluent	0.02
ST6	10 μL ST5+90 μL RNA diluent	0.002

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 RNA 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 RT-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 ST6+ NTC+sample to be tested)×3

2. Calculate the total amount of HEK293 RNA RT-qPCR MIX required for this experiment based on the number of reaction wells: HEK293 RNA RT-qPCR MIX=(number of reaction wells+2 or 3)× 4 μL (One Step RT-qPCR Reaction MIX)+(number of reaction wells+2 or 3)× 1 μL (One Step Enzyme MIX)+(number of reaction wells+2 or 3)×10 μL (HEK293 RNA Primer&Probe MIX) (2 or 3 is the operational loss).

3. Dissolve the reagents completely on ice, shake gently and mix well, centrifuge instantly, and prepare HEK293 RNA RT-qPCR MIX as shown in Table 3.

Table 3 Preparation of HEK293 RNA RT-qPCR MIX

Components	Single reaction amount
HEK293 RNA Primer&Probe MIX	10 μL
One Step qRT-PCR Reaction MIX	4 μL
One Step Enzyme MIX	1 μL
Total Volume	15 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 20 μL).

Table 4 Sample adding examples of each reaction hole

Template	Template amount	HEK293 RNA RT-qPCR MIX amount
Reference	5 μL of references ST1 to ST6	15 μL
Template-free NTC	5 μL of each RNA diluent	15 μL
Sample to be tested	5 μL of each sample to be tested	15 μL

- **Preparation and sample adding of IPC RT-qPCR reaction solution（Note: If a sample with added standard has already been set up, IPC can be used as an option）**

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 ST6+ NTC+sample to be tested)×3

2. Calculate the total amount of HEK293 RNA **IPC** RT-qPCR MIX required for this experiment based on the number of reaction wells： HEK293 RNA **IPC** RT-qPCR MIX=(number of reaction wells+2 or 3)× 4 μL (One Step RT-qPCR Reaction MIX)+(number of reaction wells+2 or 3)× 1 μL (One Step Enzyme MIX)+(number of reaction wells+2 or 3)×10 μL (HEK293 RNA **IPC** Primer&Probe MIX) (2 or 3 is the operational loss).

3. Dissolve the reagents completely on ice, shake gently and mix well, centrifuge instantly, and prepare HEK293 RNA **IPC** RT-qPCR MIX as shown in Table 5.

Table 5 Preparation of HEK293 RNA **IPC** RT-qPCR MIX

Components	Single reaction amount
HEK293 RNA **IPC** Primer&Probe MIX	10 μL
One Step qRT-PCR Reaction MIX	4 μL
One Step Enzyme MIX	1 μL
Total Volume	15 μL

4. Put the required reagent on ice to melt, shake slightly and mix well, and add samples as shown in Table 6 (total volume 20μL). （**Note**: **NCS** is an extracted RNA diluent）

Table 6 Sample adding examples of each reaction hole

Template	Template amount	HEK293 RNA **IPC** RT-qPCR MIX amount
NCS	5 μL of NCS	15 μL

Sample to be tested	5 μL of each sample to be tested	15 μL

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

- **RT-qPCR reaction procedure and parameter setting**

Taking the American ABI 7500 Real Time PCR System with software version 2.06 as an example.

1. Create a blank new program on the Experiment Properties page:

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

3. Create a new detection probe and create a new probe and sample in the Define Targets and Samples interface. Use Add New Targets to add new probes and edit the probe names in Target Name, naming them HEK293-RNA and HEK293-RNA-IPC respectively. Select the desired fluorescent and quenching groups in Reporter and Quencher.The fluorescent group of HEK293-RNA is selected as FAM, the quenching group is selected as NONE, the fluorescent group of HEK293-RNA-IPC is selected as CY5, the quenching group is selected as NONE, and the reference fluorescence is ROX. Use Add New Samples to add new samples and name them in the corresponding Sample name column.

4. Set the reaction procedure

Stage1	Reverse Transcription	Reps:1	55℃	15min
Stage2	Predenaturation	Reps:1	95℃	30s
Stage3	Cyclic reaction	Reps:45	95℃ 60℃	10s 34s

Stage3 is set to fluorescence collection at 60℃ for 34s.

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

- **RT-qPCR result analysis**

Taking the American ABI 7500 Real Time PCR System with software version 2.06 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 200, 20, 2, 0.2, 0.02 and 0.002 (pg/μL) , and name them ST1,ST2,ST3,ST4,ST5 and ST6 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 test result of NTC should be about 2-3 Ct values higher than the lowest point in the standard curve.
- Based on the differences between the actual sample matrix and NTC matrix, priority should be given to the results of sample spiked recovery rate; If the spiked sample is not tested, the CY5 channel Ct value of the sample is consistent with or ±1 of the CY5 channel Ct value of the NCS. If the Ct value of the CY5 channel in the sample is significantly increased compared to the Ct value of the CY5 channel in the negative control, it indicates that the sample may have significant inhibition.

【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. After sample pretreatment, DNase treatment is required to eliminate the influence of gDNA on detection.

3. Because RNA is easily degraded by RNA enzymes, the use of RNA enzymes should be avoided in experiments and operating environments involving RNA. If the plasmid extraction process involves the use of RNA enzymes, RNA detection should avoid the relevant operating environment.

4. It is recommended to use all components in the reagent kit after melting in a low-temperature environment, and ensure that they are fully dissolved. Before use, each component should be thoroughly mixed to ensure the uniformity of the reagent. The mixed reagent should be briefly centrifuged to concentrate all the liquid on the tube wall and lid at the bottom of the tube.

5. It is recommended to perform the operation on ice.

6. The preparation environment of negative reagents and positive reagents should be divided into different areas, and cannot be operated in one area.

7. 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.

8. 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.

9. 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.

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

11. 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.

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