Instructions for Use of *E.coli* Residual Total RNA Detection Kit (RT-qPCR)

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG-ER001

Introduction

The E.coli Residual Total RNA Detection Kit (RT-PCR) is designed for the quantitative detection of residual E.coli total RNA in various biological products to improve control quality of nucleic acid.

This kit adopts the principle of the RT-PCR fluorescent probe, combining reverse transcription PCR technology and fluorescent probe method, to realize one-step quantitative detection. With the specific primers and probes targeting RNA targets with high *E. coli* content and stable expression, the total residual RNA can be detected by absolute quantification method.

Specification

100 Reactions

Kit components

Table 1: Kit components and storage conditions

Components	Vial	Volume	Storage temperature
One Step RT-qPCR buffer	1	1 mL	-20℃
One Step Enzyme MIX	1	100 μL	
E.coli RNA Primer& Probe MIX	1	370 μL	
E.coli RNA quantitative standard	1	25 μL	
RNA IPC Primer&Probe Mix	1	370 μL	
ROX High	1	50 μL	
ROX Low	1	50 μL	
RNA diluent	2	1 mL	

Product storage conditions and shelf life

The product should be stored and transported at -20℃ while being protected from light; the shelf life is 18 months.

Applicable model

Including but not limited to ABI7500, BioRad CFX96, Bioer FQD-96A, Roche Light Cycler 480 and other real-time quantitative fluorescence PCR instruments. (Table2)

Instruments	ROX reference stain
Applied Biosystems® 5700, 7000, 7300, 7700, 7900HT, StepOne™, and StepOnePlus™	ROX High
Applied Biosystems® 7500, ViiA™ 7, QuantStudio™ 12K Flex, Agilent Mx3000P™, Mx3005P™, and Mx4000™	ROX Low
Rotor-Gene™, DNA Engine Opticon™, Opticon™ 2, Chromo 4™ Real-Time Detector, Mastercycler® ep realplex, Smart Cycler®, Roche LightCycle®480, Roche LightCycler®Nano, Bio-Rad CFX96, and IIlumina Eco™	No ROX

Note:Select an appropriate ROX for the model. If the corresponding model is not found in the above table, please consult our company or the instrument manufacturer

Consumables and equipment to be self-prepared

Please prepare the following consumables and equipment before the test

- 1.5 mL or 2 mL sterile low adsorption centrifuge tubes
- 96-well qPCR plate or 8-strip tube adapted to PCR instrument
- 1000 μL, 200 μL, and 10 μL sterile low adsorption pipette tips with cartridge
- Fluorescence quantitative PCR instrument
- Pipettes of various specifications (e.g., 1000 μL, 200 μL, 10 μL, 2.5 μL)
- Centrifuge
- Oscillator
- Magnetic stand
- Water bath/metal bath

Test steps

I．Sample Preprocessing（need to use our kit：HG-CL300）

1. The detection of residual E. coli RNA in the sample is a residual detection item, and DNA in the test sample needs to be removed before detection. Therefore, sample preprocessing is required for the test sample. The sample preprocessing kit provided by our company can be selected, and the processing method is as follows:

Table 3　Preprocessing reaction system

Components	Reaction in single-well (μL)
DNase I	1
10X Reaction Buffer with $MgCl_2$	2
RNase Inhibitor	0.5
Enzyme free deionized water	12.5
Sample	4
Total volume	20

2. Prepare the test sample DNase I digestion solution according to the above table, shake and mix well, and centrifuge for 5 s；

3. Incubate the prepared test sample DNase I digestion solution in a 37℃ thermostat water bath for 60 min；

4. Take out the test sample DNase I digestion solution after incubation, centrifuge it for 5 s, and incubate it in a 65℃ dry thermostat for 10 min to inactivate DNase I. Store the inactivated test sample DNase I digestion solution in a refrigerator at 2-8℃ for temporary storage.

II．qPCR Operation Steps

1. Dilution of Reference Standard of E Coil Genome RNA

1.1 Quantitative reference: Take out E.coli RNA quantitative reference and RNA diluent, and thaw on ice; after thawing completely, shake gently to mix well, and centrifuge instantly;

1.2. Take 6 clean 1.5 mL centrifuge tubes and label them as ST0, ST1, ST2, ST3, ST4, and ST5, respectively;

1.3. The standard dilution process is shown in the table below:

Table 4

Standard No.	Dilution volume	Concentration (fg/μL)
ST0	5μL Quantitative reference + 45 μL DNA diluent	2.00E+05
ST1	5 μL ST0 + 45 μL DNA diluent	2.00E+04
ST2	5 μL ST1 + 45 μL DNA diluent	2.00E+03
ST3	5 μL ST2 + 45 μL DNA diluent	2.00E+02
ST4	5 μL ST3 + 45 μL DNA diluent	2.00E+01
ST5	5 μL ST4 + 45 μL DNA diluent	2.00E+00

2. Preparation and addition of RT-qPCR reaction solution

2.1 Take out each reagent from the freezer and place them on ice:

2.2 Preparation of PCR reaction solution：See Table 5 for details, One Step RT-qPCR buffer、One Step Enzyme Mix、E.coli RNA Primer& Probe Mix、ROX，These four reagents can be premixed into Mix in advance, and then 5 μL of Standard curve (ST1/ST2/ST3/ST4/ST5), NTC, and test solution are added successively and mixed well. Three replicates are prepared for each sample.

Table 5　RT-qPCR MIX Preparation

Components	Volume required for single reaction(μL)	Note
One Step RT-qPCR buffer	10	Mix can be premixed in advance and 15 μL of the mix can be added to each well and 5 μL sample/Standard curve/NTC can be added correspondingly.
One Step Enzyme Mix	1	
E.coli RNA Primer& Probe Mix	3.6	
ROX	0.4	
Standard curve /NTC/sample	5	
Total volume	20	

* Please select appropriate ROX for corresponding model. If there is no ROX suitable for the model, please add deionized water (free of nucleic acid and nuclease contamination) of same volume.

2.3 Calculate the total volume of MIX required for this analysis based on the number of reaction wells.

MIX = (number of reaction wells + 2)× (10+1+3.6+0.4) μL (including volume loss of 2 wells)

After filling the sample and sealing the tube, please centrifuge at low speed for 10 sec to collect the liquid on the tube wall to the bottom of the tube, then shake and mix well for more than 5 sec to completely mix the reaction mixture, then centrifuge the tube at low speed for 10 sec to collect the liquid on the tube wall to the bottom of the tube, and exhaust the bubbles if there are bubbles.

3. Preparation and sample addition of reaction mixture prepared by IPC group (IPC detection can be cancelled if there is already a way such as spike and recovery in the detection)

Each experiment requires a template free control (IPC-NTC) and IPC (IPC-S) detection for each test sample, according to Table 6:

Table 6. IPC RT-qPCR MIX Preparation

Components	Volume required for single reaction(μL)	Note
One Step RT-qPCR buffer	10	Mix can be premixed in advance and 15 μL of the mix can be added to each well and 5 μL IPC-NTC/ IPC-sample can be added correspondingly.
One Step Enzyme Mix	1	
RNA IPC Primer&Probe Mix	3.6	
ROX	0.4	
IPC-NTC/ IPC-sample	5	
Total volume	20	

Notes:

Spiking System ERC: 90 μL sample + 10 μL ST3;

Spike recovery = ERC/(0.9* sample + 0.1ST3); (Spike recovery QC criteria: 50% to 150%)

Select between spike recovery and IPC, and IPC-S in Table 7 can be replaced with ERC-S if spike recovery is selected.

4. Layout illustration of reaction wells

Table 7. Layout illustration of Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	ST1	ST1	ST1							S1	S1	S1
B	ST2	ST2	ST2							S2	S2	S2
C	ST3	ST3	ST3							S3	S3	S3
D	ST4	ST4	ST4									
E	ST5	ST5	ST5									
F										IPC-S1	IPC-S1	IPC-S1
G					NTC	NTC	NTC			IPC-S2	IPC-S2	IPC-S2
H					IPC-NTC	IPC-NTC	IPC-NTC			IPC-S3	IPC-S3	IPC-S3

Note: This example represents the detection including 1 NTC, 3 test samples, and a DNA standard curve with 5 concentration gradients. For IPC-NTC and IPC of each sample (IPC-S1, IPC-S2, and IPC-S3), prepare wells in triplicate. During actual testing, samples in the 96-well plate may be loaded based on the actual number of test samples, as per the plate layout in Table.

Seal the 96-well plate with an optical plate sealer, gently shake to mix well, perform rapid centrifugation with a centrifuge dedicated for 96-well plate to let all liquid gather at tube bottom, then place the plate in the qPCR system

III. Reaction program and parameter setting

For testing on qPCR system, taking the combination of 7500 qPCR system (ABI) and software version v2.0.6 as an example.

1. Create a new blank program, select the absolute quantitative detection template and Taqman probe method.

2. Create a new probe named "E. coli RNA", select FAM for the reporting fluorescent group and "none" for the quenching fluorescent group; create a new probe named "RNA IPC", select VIC for the reporting fluorescent group, and "none" for the quenching fluorescent group; the reference fluorescence of the detection is ROX.

3. Set up a three-step reaction program like Table 8: the reaction volume of 20 μL.

Table 8. reaction program

Cyclic Steps	Temperature	Time	Cyclic number
Reverse transcription	50℃	15min	1
Pre-denaturation	95℃	30 sec	1
Denaturation	95℃	10sec	45
Annealing/extension (collecting fluorescence)	60℃	40 sec	

Note: For other models of equipment, if you encounter any problems, you can consult our company or the instrument manufacturer.

4. Run the PCR program.

IV. Result analysis

1. In the "Amplification Plot" of Results, the user may initially check whether the amplification curve is of normal shape. Usually, the system will automatically set the threshold and baseline. Multiple thresholds may be generated in case of different target settings, leading to inaccurate result analysis. In such case, please manually set the threshold line, which must be within the exponential amplification region, for example, the threshold is usually set to 0.15. Select "Auto Baseline", and click "Analyze" to view the adjusted results.

2. In the "Standard Curve" of Results, the slope, intercept, and R^2 of the standard curve can be read. Acceptable standard effective : 85%~110%，R^2≥0.98.

3. In the "Report" in "Results", the detection values (in fg/μL) of NTC and test samples can be read in the "Mean Quantity" column. For NTC, the Ct value should be greater than that of ST5, with a difference being no less than 2, and the detection value should be lower than 2 fg/μL.

4. Analyze the Ct value of IPC. Normally, the Ct-IPC value of the test sample should be consistent with that of NTC, or within in the range of Ct-IPC value of NTC ± 2. If the Ct-IPC value of the test sample is significantly higher than that of NTC, it indicates that there may be significant inhibition in the test sample.

5. Calculation of *E.coli* RNA residue：

Abnormal data and unexpected situations in the detection process, such as tube leakage, sample evaporation and sample addition error, should be deleted and the reasons should be explained in the detection record. Other normal data can continue to be used in the calculation of detection results.

$$\textit{E.coli}\ \text{RNA residue (fg/ng DNA)} = \frac{\textit{E.coli}\ \text{RNA residue detection value (fg/μL)}}{\text{Sample concentration (ng/μL)} * 5}$$

Precautions

1. The kit has been validated for the stability (freezing-thawing and other factors) and does not require dispensing.
2. The preparation for negative samples and positive samples (reference and samples to be tested, etc.) should be separated into different environments and should not be operated in one area. The preparation personnel should wear neat masks, gloves and cleanroom garment.
3. Tips shall be changed between different loading steps in time to avoid cross-contamination and long-time opening.
4. The kit must be used within the shelf life.0
5. All components in the kit are recommended to be used after melting in a low temperature environment.
6. The best detection effect can be ensured only by strictly following the instructions and using all the reagents provided with this kit.
7. Subsequent qPCR detection shall be performed immediately after sample preprocessing and purification as far as possible on the same day to ensure the accuracy of test results.
8. The final test results are closely related to the efficacy of reagents, operator's operation methods and test environment.
9. Our company is only responsible for the kit itself and not for the sample consumption caused by the use of the kit. Users should fully consider the possible usage of sample before use and reserve sufficient samples.
10. This kit is for in vitro research use only and is not used for clinical diagnosis.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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